review

Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression

Kristina Schoonjans, Bart Staels, and Johan Auwerx[1]

U.325 INSERM, Département d'Athérosclérose, Institut Pasteur de Lille, 1, rue du Prof. Calmette, 59019 Lille Cedex, France

Abstract The three types of peroxisome proliferator-activated receptors (PPAR), termed α, δ (or β), and γ, belong to the nuclear receptor superfamily. Although peroxisome proliferators, including fibrates and fatty acids, activate the transcriptional activity of these receptors, only prostaglandin J_2 derivatives have been identified as natural ligands of the PPARγ subtype that also binds thiazolidinedione antidiabetic agents with high affinity. PPARs heterodimerize with retinoic X receptor (RXR) and alter the transcription of target genes after binding to response elements or PPREs, consisting of a direct repeat of the nuclear receptor hexameric DNA recognition motif (PuGGTCA) spaced by 1 nucleotide (DR-1). Upon activation by fatty acids (FAs) and drugs that affect lipid metabolism, PPARs control the expression of genes implicated in intra- and extracellular lipid metabolism, most notably those involved in peroxisomal β-oxidation. PPARs partially mediate the inductive effects of fibrates and fatty acids on high density lipoprotein (HDL) cholesterol levels by regulating the transcription of the major HDL apolipoproteins, apoA-I and apoA-II. The hypotriglyceridemic action of fibrates and certain fatty acids also involves PPAR and is constituted of: *1)* increased hydrolysis of plasma triglycerides due to induction of LPL and reduction of apoC-III expression; *2)* stimulation of cellular fatty acid uptake and conversion to acyl-CoA derivatives due to increased expression of genes for fatty acid transport protein and acyl-CoA synthetase; *3)* increased peroxisomal and mitochondrial β-oxidation; and *4)* decreased synthesis of fatty acids and triglycerides and decreased production of very low density lipoprotein (VLDL). Hence, both enhanced catabolism of triglyceride-rich particles and reduced secretion of VLDL particles contribute to the hypolipidemic effect of fibrates and fatty acids. Finally, PPARs appear to be involved in differentiation processes because activation of PPARγ2 triggers adipocyte differentiation and stimulates expression of several genes critical to adipogenesis. It is suggested that PPARs are key messengers responsible for the translation of nutritional and pharmacological stimuli into changes in gene expression and differentiation pathways.–**Schoonjans, K., B. Staels, and J. Auwerx.** Role of the peroxisome proliferator activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* 1996. **37:** 907–925.

Supplementary key words hypolipidemic drugs • lipid metabolism • nuclear hormone receptors • RXR • transcription

PPARs, NOVEL NUCLEAR RECEPTORS

Peroxisome proliferation involves the nuclear receptor PPAR

Peroxisome proliferation is a pleiotropic cellular response to a range of chemical compounds (including fibrates, phthalate and adipate ester plasticizers, herbicides, leukotriene antagonists, certain natural and modified fatty acids, acetylsalicylic acid) and to certain pathophysiological conditions involving drastic changes in both cellular morphology and enzymatic activity (for review, see refs. 1–3). The phenomenon is seen primarily in liver and kidney and furthermore is organism-specific, because rodents are more susceptible to peroxisome proliferators than other organisms. Sustained peroxisome proliferation in rodents often leads to hepatocarcinogenesis (2, 4). In addition, hormonal and nutritional factors (3, 5) are important modulators of the response to peroxisome proliferators, illustrating the complexity of this phenomenon.

Peroxisome proliferators induce numerous alterations in hepatic lipid metabolism. This led to the development of the substrate overload hypothesis. This hypothesis provides a link between the observed perturbation in lipid metabolism and peroxisome proliferation. According to this hypothesis, an increased

Abbreviations: ACO, acyl-CoA oxidase; aP2, adipocyte fatty acid binding protein P2; apo, apolipoprotein; C/EBP, CCAAT enhancer binding protein; DR-1, direct repeat spaced by one nucleotide; HDL, high density lipoprotein; Lp AI, HDL containing apoA-I only; Lp AI:AII, HDL containing apoA-I and apoA-II; LPL, lipoprotein lipase; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; RAR, retinoic acid receptor; RE, response element; RXR, retinoic X receptor (9-*cis* retinoic acid receptor); TG, triglyceride; TR, thyroid hormone receptor; VLDL, very low density lipoprotein.

[1]To whom correspondence should be addressed.

flux of fatty acids to the liver stimulates a series of metabolic changes (see ref. 1 for review), ultimately leading to the accumulation of long-chain dicarboxylic acids which, in turn, stimulate peroxisomal β-oxidation. This hypothesis, however, cannot explain the extremely rapid induction of gene transcription after peroxisome proliferators. These discrepancies, together with the identification and characterization of a peroxisome proliferator-binding protein in rat liver (6), led to the receptor hypothesis, which is complementary to the substrate overload hypothesis. In 1990, the first peroxisome proliferator-activated receptor (PPAR) was cloned from mouse liver by Isseman and Green (7), soon followed by other PPAR homologs in several species (8–16). Unequivocal evidence for a direct implication of PPARα in peroxisome proliferation was recently obtained. Mice with a targeted disruption of the PPARα gene are resistant to peroxisome proliferation (17). The discovery that a nuclear receptor can trigger peroxisome proliferation through a transcriptional mechanism has opened a completely new field of research and is consistent with dietary factors being important modulators of gene expression.

General properties of the nuclear hormone receptors

In order to survive, organisms develop efficient mechanisms to permit appropriate adaptation to extra- and intracellular signals, such as hormones and diet-derived factors. This adaptation process is often mediated via receptors. Two receptor-mediated pathways can be distinguished, depending on the property of the ligand. Hydrophilic ligands generally bind to receptors on the surface of the plasma membrane and activate a cascade of second messengers. These messengers eventually transmit a signal to *trans*-acting factors that control transcription rates of target gene(s). Hydrophobic signalling molecules, such as certain hormones and vitamins, enter the cell by simple or facilitated diffusion and transduce their signal to the genome via intracellular receptors (18–20). These intracellular ligand-activated receptors or nuclear (hormone) receptors act as transcription factors and exert their regulatory functions directly at the gene level. This group of nuclear receptors represents the largest family of transcription factors in eukaryotes.

The simplest classification distinguishes two major subgroups of nuclear receptors according to their cellular localization in the unliganded form (19). The first subgroup (type I receptors) consists of receptors that are localized in the cytoplasma and, upon binding of their ligand, are translocated to the nucleus. In the inactive form, these receptors usually are associated with heat-shock proteins. The classic steroid receptors such as the glucocorticoid, androgen, progesterone, mineralocorticoid, and estrogen receptors belong to this group. The nuclear localization of the second subgroup (type II receptors) of receptors is independent of ligand binding. After binding of ligand in the nucleus, their conformation changes such that transactivation of target genes is promoted. In contrast to the classic steroid receptors, type II receptors can bind to DNA in the absence of ligand. This group includes the thyroid (TR), retinoic acid (RAR), 9-*cis* retinoic acid (RXR), vitamin D (VDR), and peroxisome proliferator-activated (PPAR) receptors. Most of the orphan receptors, a growing group of receptors, without known ligand, also belong to this group (21). Beside the differences in ligand binding and cellular localization, the overall structures and functions of type I and II receptors are similar (19).

General properties and structure of PPAR

The DNA binding domain is highly conserved among all members of the nuclear receptor superfamily and permits the cloning of many new members of this family. Most of the new receptors are orphan receptors and the various PPARs are among the best characterized of these. First to be cloned was mouse (m) PPARα (7), so named because it is activated by peroxisome proliferators. To date, three different types of PPARs, α, δ (NUC I, FAAR, β), and γ have been identified in different vertebrates. The α, β, and γ types have been cloned from *Xenopus* (x); from humans and mouse, α, δ, and a γ types have been cloned (7, 8, 10–13, 16, 22, 23). The α form has been cloned from rat and the γ form from hamster (9, 24). Mouse PPARγ has two isoforms, γ1 (12) and γ2 (13, 25), which differ only in their N-terminal 30 amino acids, and are derived from the same gene by alternative promoter usage and splicing (26, 27). Differential splicing of the first exon also has been reported for mPPARα (28), and in retinoid and thyroid hormone receptors (29). The genomic structure for mPPARα (28), mPPARγ (27), and xPPARβ (30) have been determined and they are similar in organization to other nuclear receptors.

Based on a phylogenetic analysis of these genes, PPARs form a distinct subfamily of nuclear receptors (31). PPARs have so far only been identified in vertebrates; a *Drosophila* PPAR homolog has not been found. The closest *Drosophila* relative is the orphan receptor E75. In vertebrates, the DNA binding domain of the orphan receptor Erb-a related protein 1 (EAR-1) exhibits the highest degree of homology with the DNA binding domain of the PPARs (31). A phylogenetic analysis based on the ligand binding domain showed that the PPAR ligand binding domain was most closely related to the rat liver-derived receptor 1 (RLD-1) or liver X receptor (LXR) (32, 33), another orphan receptor.

Like the other nuclear receptors, PPARs have a modu-

lar structure consisting of six functional domains, A/B, C, D, and E/F. A limited number of studies on the structure–function relationship of PPARs has been performed, and the discussion below is based on the conservation of the domain structure amongst the different nuclear receptors. The C or DNA binding domain of PPAR contains about 66 amino acids and targets the receptor to specific DNA sequences or response elements (REs). The DNA binding domain is stabilized by zinc atoms, each binding to four invariant cysteine residues. Each of these zinc finger complexes is followed by an α-helical structure (**Fig. 1**); one of them serves as the major recognition helix that makes base-specific contacts within the major groove of the core site (34, 35). Two conserved sets of functionally important amino acid residues have been identified in the DNA binding domain. The first, termed the P-box, overlaps the second knuckle of the first zinc finger and determines specific contacts between receptor and DNA. The P-box of PPAR is identical to those of TR, VDR, RAR, RXR, Neural Growth Factor 1-B (NGF-1B, NURR-1), EAR-1 (or Rev-ErbAα), Ecdysone Receptor, E75, and Ultraspiracle (Usp). The second conserved box, the D-box, consists of amino acid residues of the first knuckle of the second zinc finger, and may be involved in protein–protein interactions, such as receptor dimerization (18, 36). The PPAR D-box differs from that of other nuclear receptors because it is composed of three rather than five amino acids. So far, the only other exception to the "five" rule, is the *Drosophila* receptor *tailless (tll)* and its vertebrate homologue Tlx (37), each of which have seven amino acids in their D-boxes.

The relatively large E or ligand-binding domain of nuclear receptors is a multifunctional domain. In addition to ligand binding, it is involved in dimerization, nuclear localization, ligand-dependent transactivation, intermolecular silencing (e.g., for TR, RAR, COUP-TF, etc.) and intramolecular repression (e.g., progesterone receptor), and association with heat-shock proteins (reviewed in refs. 18, 19, 36 and references therein). The dimerization region of PPAR is contained in a region of nine heptad repeats that shows some similarity to the leucine zipper sequence mediating c-fos/c-jun dimerization (38, 39). Although the activation domains of PPAR are not experimentally characterized, it is likely that they will have characteristics similar to those described below for the other nuclear receptors. In addition to the constitutive activating function 1 (AF-1) localized in the A/B domain, nuclear receptors contain an amphipathic α-helical ligand-dependent activating domain localized in the C-terminal part of the E-domain termed AF-2 (40). Depending on the promoter, AF-1 and AF-2 may act independently or cooperatively (41). The ligand-binding domain in nuclear receptors thus functions as a modular unit whose transcriptional activities are controlled by ligand-binding and dimerization. The ligand-induced conformational change may unmask the major dimerization region in the ligand-binding domain (42–45). Therefore, dimerization and ligand-binding would confer transcriptional activities on the heterodimeric receptors that are distinct from those of the component monomers. This could favor high affinity binding and transactivation of the responsive gene (33, 46, 47). This arrangement permits a limited number of receptors to generate a diverse set of transcriptional responses to multiple hormonal signals. In comparison to the retinoid receptor subfamilies, the ligand-binding domains of the different PPARs are less conserved; this may indicate that they bind similar but not identical ligands.

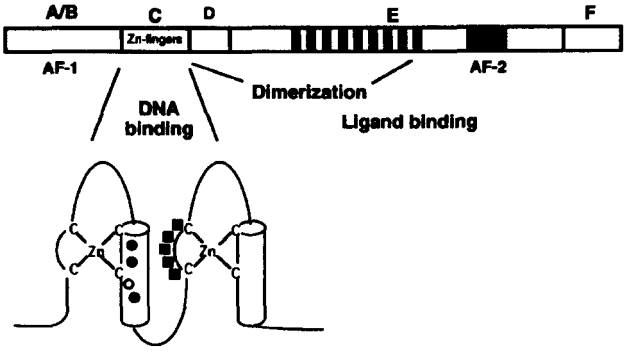

Fig. 1. Functional domains of the nuclear hormone receptors. The activation domains AF-1 and AF-2 are indicated. The nine heptad repeats are indicated by the 9 black stripes in the E domain. The last heptad repeat is involved in dimerization. The schematic structure of the two zinc fingers in the DNA binding domain of the nuclear receptors is depicted under the C-domain. The P-box is composed of the amino acid residues depicted by circles and the two flanking cysteine residues. The amino acids in the D-box are indicated by squares. Residues in black determine the specificity of the P-box regarding half-site recognition or of the dimerization interface and spacing between the half-site (D-box).

Tissue-specific, developmental, and regulated expression of PPAR

The presence of multiple PPARs raises questions about their physiological function. A first attempt to answer this question consists in defining their tissue specificity and relative abundance. Analysis of PPAR mRNA distribution indicated that the mammalian PPARα is predominantly expressed in liver, heart, kidney, intestinal mucosa, and brown adipose tissue, tissues with high catabolic rates for fatty acids and peroxisomal metabolism (7, 48, 49). PPARδ also is abundantly and ubiquitously expressed, whereas PPARγ presents a much more restricted expression (49). Although mPPARγ1 is rather ubiquitously expressed, mPPARγ2 is predominantly expressed in adipose tissue (12, 13, 26). In *Xenopus* the expression of the various PPAR forms is different from that in mammals (8, 31, 50).

Both xPPARα and xPPARβ are detected during oogenesis, whereas xPPARγ is absent (8). During mouse embryogenesis, mPPARδ expression precedes the onset of expression of mPPARα and mPPARγ (14). Although it has been reported that PPARα mRNA levels are induced in rat liver by fenofibrate administration (51), this induction could not be confirmed either in vivo after administration of fenofibrate to rats or in vitro after addition of fenofibric acid to primary rat hepatocytes (B. Staels and J. Auwerx, unpublished data). Interestingly, PPARα gene expression in rat liver is strongly induced at the transcriptional level by glucocorticoids (52, 53), an effect that is attenuated by insulin (53). The regulation of the PPARα gene by glucocorticoids has been linked to a diurnal rhythmicity in PPARα expression and its induction in response to stress (54). Thus PPARα, a major regulator of metabolic pathways, is itself subject to transcriptional control by other hormones.

Activators of PPAR

Several exogenous substances, such as hypolipidemic drugs, phthalate ester plasticizers, and herbicides, activate PPARs (7, 8), but evidence demonstrating that these compounds are ligands for PPAR is still lacking. As high fat diets (55–57) and (patho)physiological conditions characterized by a fatty acid overload (58, 59) stimulate peroxisomal β-oxidation and peroxisome proliferation, fatty acids have been proposed to be the endogenous PPAR activators. Indeed, a broad range of fatty acids are capable of activating the different PPARs (9–11, 50, 60). With the exception of short-chain fatty acids ($< C_{10}$), very long-chain monounsaturated fatty acids and dodecanedioic acid, all fatty acids activate PPAR to a certain degree in transient assay systems. Unlike natural fatty acids, which are good substrates for β-oxidation, substituted fatty acids, which are not a substrate for β-oxidation, are more potent PPAR activators, suggesting that the degree of PPAR activation is inversely correlated with the rate of fatty acid β-degradation. Although these studies demonstrate that exogenous fatty acids activate PPAR, it is not clear whether the active form is the acid or the acyl-CoA thioester, the production of which is controlled by the enzyme acyl-CoA synthetase. These acyl-CoA derivatives are either stored as an intracellular acyl-CoA ester pool complexed with the acyl-CoA binding protein or utilized in various intracellular enzymatic pathways. The direct involvement of acyl-CoA derivatives in intracellular lipid metabolism (in contrast to free fatty acids), together with the finding that xenobiotic peroxisome proliferators also form acyl-CoA esters (61), suggests that acyl-CoAs may be PPAR activators and/or ligands. This hypothesis is supported by the finding that in prokaryotes acyl-CoA derivatives are the ligands of the transcription factor, FadR, which regulates the expression of genes involved in FA transport and β-oxidation (62). However, a report comparing the efficiency of activation by free acids and their corresponding acyl-CoA derivatives in transient expression assays concludes that the immediate inducer is the free acid (63).

Thiazolidinedione antidiabetic agents (such as the compound BRL49653) bind selectively and with high affinity (K_d of 40 nM) (64) and potently activate the PPARγ subtype (64), suggesting that they are ligands. Although thiazolidinediones are high affinity synthetic ligands for PPARγ, arachidonic acid metabolites of the prostaglandin J2 group are the natural activators (65) and ligands (66, 67) for this receptor, confirming experimentally that fatty acid derivatives are the endogenous ligands of one PPAR type.

Some receptors also can be activated by ligand-independent mechanisms (68–71), suggesting that the intracellular and membrane signalling pathways are not completely separated and that cross-talk may have important implications. However, such activation pathways have not yet been described for PPARs.

PPREs: hormone response elements that have the potential for multiple cross-talk

The cognate DNA sequences to which receptors bind are designated (hormone) response elements (REs). REs are characterized by a high degree of conservation; this is not surprising, considering the highly conserved structure of the DNA binding domain of the nuclear receptors. Based upon homology in the P-box sequence, an indicator of DNA recognition specificity, the receptors can be classified into several groups (19). Although all nuclear receptors recognize derivatives of a canonical hexameric DNA recognition motif (PuGGTCA), mutation and duplication (leading to distinct relative orientations and spacing of repeats of this motif) and addition of flanking sequences have generated distinctive REs specific for the various receptors. Most receptors bind to DNA as dimers. Type I receptors, such as the glucocorticoid receptor, bind as homodimers to palindromic REs. Type II receptors bind preferentially as heterodimers to direct repeats, but some of them also bind as homodimers. In addition to the orientation of the half sites, the number of nucleotides spacing the two half sites determines the combination and identity of receptors that will bind to a RE. The "3, 4, 5 rule" tries to generalize the identity of the dimeric receptor-complex in function of the number of spacer nucleotides (72, 73). Although this rule is generally correct, receptors may bind promiscuously and activate other REs, albeit with lower efficiency (74, 75). This binding flexibility of nuclear receptors may permit more complicated and subtle regulatory mechanisms. Finally, it must be men-

tioned that not all receptors function as dimers because the orphan receptors, EAR-1, Rev-erbβ (RVR), Neural Growth factor-1 (NGF1-B, NURR-1), *Fushi Tarazu* factor 1 (FTZ-F1) α,β, Steroidogenic Factor-1 (SF-1), Embryonal long terminal repeat-binding protein (ELP), and Retinoic Z Receptor α, β, (RZR, also termed Retinoic O receptor or ROR) exert their effects on gene transcription by binding as monomers to a single hexameric core

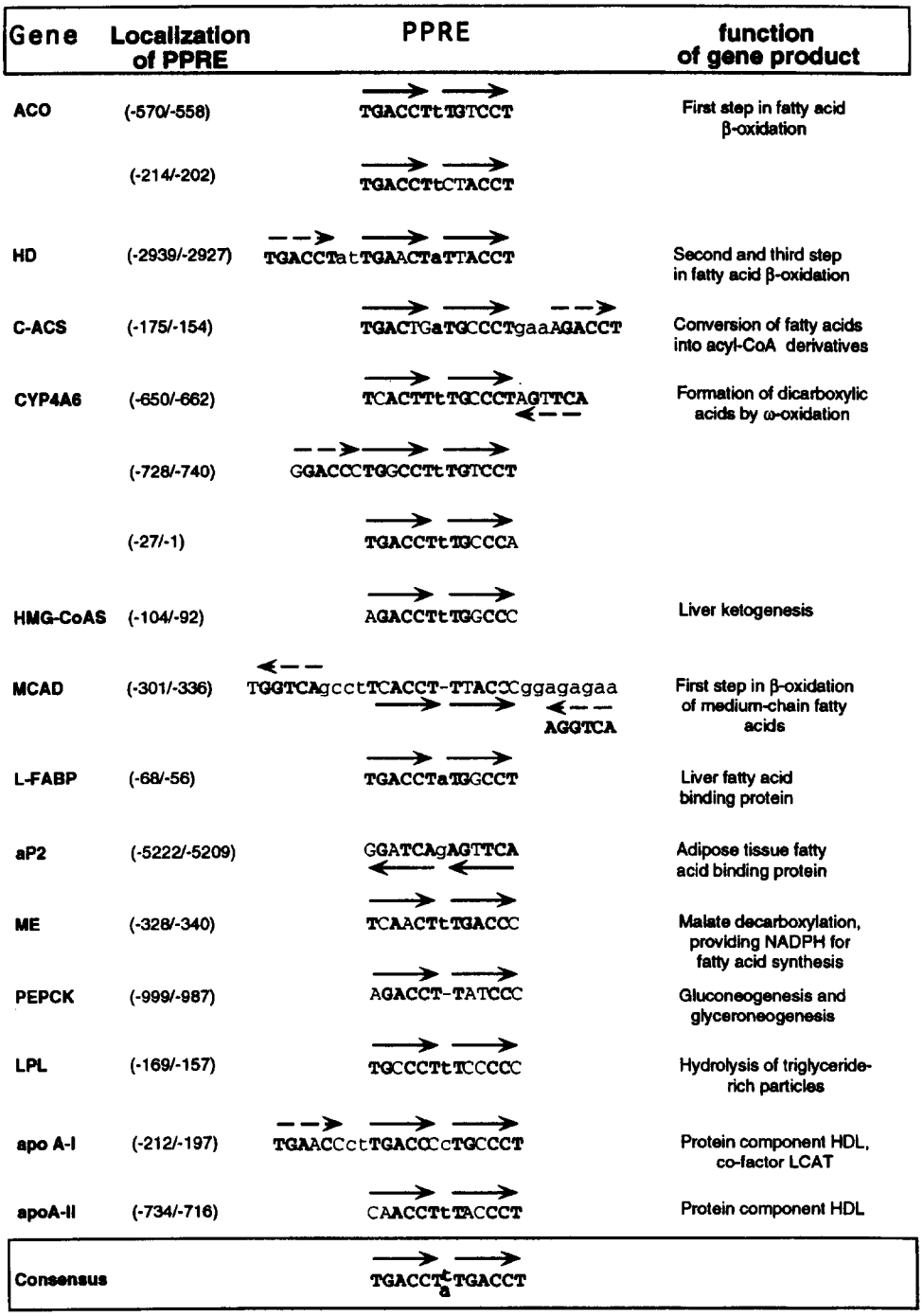

Fig. 2. Functional PPREs. The DR-1s are indicated by a solid arrow which is indicated above the sequence when the coding strand is depicted; a dotted arrow indicates eventual additional half sites located adjacent to the DR-1 element. Abbreviations used in this figure include: ACO, acyl-CoA oxidase; ACS, acyl-CoA synthetase; aP2, adipocyte fatty acid binding protein P2; apo, apolipoprotein; L-FABP, liver fatty acid binding protei˜; HD, enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase; HMG-CoAS, HMG-CoA synthase; LPL, lipoprotein lipase; MCAD, medium-chain acyl-CoA dehydrogenase; ME, malic enzyme.

motif (76–83), flanked by specific sequences upstream of the half site (78, 82). Amino acid residues carboxy-terminal to the DNA binding domain (A-box) are required for binding to the extended half site (84). These nuclear receptors may define a third class of receptors (type III), the monomeric nuclear receptors. Some promiscuity may exist between the prototypic monomeric receptors because NGF1-B/NURR-1 heterodimerizes with RXR (47, 85).

PPARs possess the same P-box sequence, CEGCKG, as most of the type II receptors that heterodimerize and recognize direct repeats of AGGTCA (or TGACCT) motifs with a variable number of intervening nucleotides. As a consequence, the peroxisome proliferator RE (PPRE) should consist of a direct repeat rather than a palindrome or inverted repeat. Indeed, most of the PPREs so far characterized are direct repeats spaced by one intervening nucleotide (DR-1) (**Fig. 2**). RXR acts as the preferential second partner in a functional PPAR-containing dimeric receptor complex (**Fig. 3**). In fact, Kliewer et al. (86) first demonstrated that PPAR and RXR interact synergistically and in a ligand- or activator-dependent way on different DR-1s and confers peroxisome proliferator responsiveness to a heterologous acyl-CoA oxidase (ACO)-PPRE driven promoter. Soon after, the cross-talk between the PPAR and RXR signalling pathways was confirmed in the natural ACO promoter context (50, 60), indicating convergence of retinoic acid and peroxisome proliferator signalling pathways. This convergence between PPAR/RXR signalling pathways is physiologically relevant because in rat hepatocytes, peroxisome proliferators and retinoic acid independently induce the expression of the PPAR target genes, ACO and acyl-CoA synthetase; they act synergistically when added together (86, 87 and unpublished data).

In addition to the classical peroxisome proliferators, hormones such as dehydroepiandrosterone and dexamethasone, or conditions such as hyperthyroidism and vitamin E deficiency, also induce peroxisomal β-oxidation (3). Except for dexamethasone, which induces peroxisome proliferation indirectly by increasing PPARα expression (52, 53), the mechanism by which these compounds or conditions interact with the PPAR signalling pathway remains to be determined. These hormones or conditions do not activate PPAR directly, so it is assumed that they act indirectly on the PPAR/RXR signalling pathway (similar to dexamethasone) or through convergence of the PPAR signal with those of other hormone receptors. Functional interaction between PPAR and other receptors is a potential mechanism for the sex-, species-, and tissue-specific differences in peroxisome proliferation.

So far, RXR is the only heterodimeric partner for PPAR that has been proven to be functionally involved in the peroxisome proliferator signalling pathway. It has been reported that PPAR can act as a coregulatory factor for TR in thyroid hormone signalling (88). PPAR cooperates in TRβ-specific signalling through a newly identified DR-2 element. In addition, PPAR antagonizes TRα and TRβ action via the TR consensus DR-4 target site by forming inactive complexes. Hence, PPAR may posi-

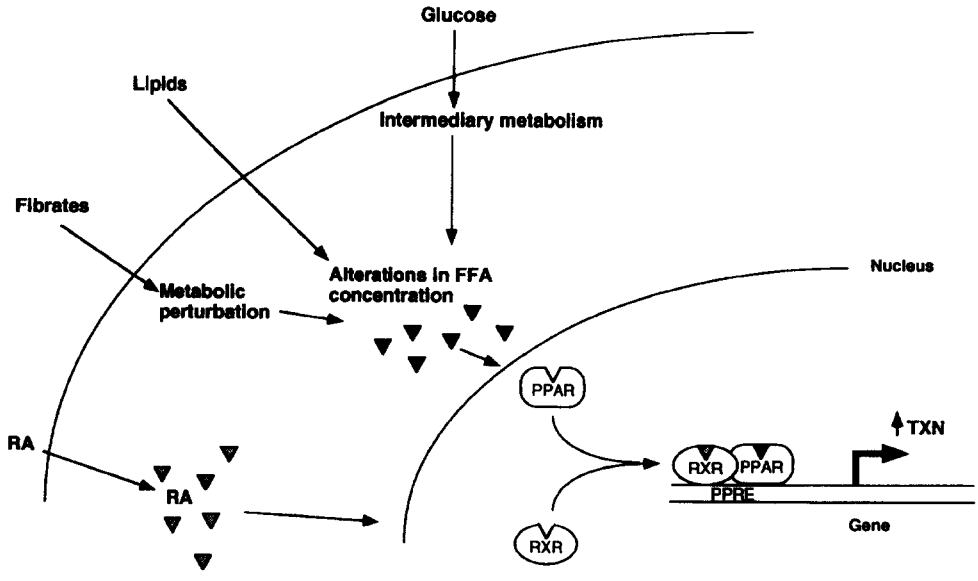

Fig. 3. Scheme summarizing the activation of the PPAR signalling pathway by multiple physiological stimuli and describing its cross-talk with the retinoic acid signalling pathway. Abbreviations: TXN, transcription.

tively or negatively influence thyroid hormone action, depending on the nature of the TR-RE (DR-2 or DR-4) and the TR type (TRα or TRβ). These in vitro data may have physiological relevance because both peroxisome proliferators and thyroid hormone have calorigenic effects and both have comparable stimulatory effects on enzymes involved in lipid metabolism, such as mitochondrial glycerol-3-phosphate dehydrogenase and S14, classically considered to be thyroid hormone-dependent (89). However, other thyroid hormone-regulated enzymes involved in lipid metabolism, such as malic enzyme, may be regulated by peroxisome proliferators and thyroid hormone through independent signalling pathways and REs (90).

Nuclear receptors may also regulate transcription negatively. Chicken ovalbumin upstream promoter transcription factors (COUP-TF1 = EAR-3 and COUP-TF2 = ARP-1) act as negative modulators of hormone responsive genes by competing for binding sites with their positive counterparts (91, 92). Although they have a preference for DR-1s. COUP-TFs exhibit a high degree of flexibility with respect to the sequences to which they will bind. In this context, it is interesting to note that COUP-TF1 and COUP-TF2 block peroxisome proliferator responsiveness of the multifunctional enzyme and the CYP4A6 genes, respectively (93, 94).

As discussed above, promiscuous binding of type II nuclear receptors to REs and exceptions to the spacer rule are becoming more and more numerous. PPAR is no exception. PPARs bind to and transactivate a palindromic estrogen response element with 3 spacer nucleotides, thereby conferring responsiveness to peroxisome proliferators on a heterologous estrogen response element-containing promoter (38). Furthermore, in the medium chain acyl-CoA dehydrogenase gene there is a PPRE that does not contain a DR-1 (95). As with other type II receptors, therefore, PPAR can enhance the specificity of gene regulation by expanding the combination of heterodimeric partners and by binding to REs with different numbers of spacer elements.

Target genes and functional implication of PPAR

Since the discovery of the transcription factor PPAR, scientists have been attempting to define its role in directing the peroxisome proliferator response (Figs. 2 and 4). The gene encoding the rate-limiting enzyme in the peroxisomal β-oxidation pathway, acyl-CoA oxidase (ACO), was an obvious candidate for attempting to identify a potential RE for PPAR. Promoter analysis of this gene led to the identification of two PPREs, each of which confers transcriptional activation by PPAR (8, 96, 97). A PPRE also has been characterized in the regulatory sequences of the multifunctional enzyme, the second enzyme in the peroxisomal β-oxidation pathway (98–100). Although transcription of the gene for the third enzyme of the β-oxidation cycle, ketoacyl-CoA thiolase, is also stimulated by peroxisome proliferators, a functional PPRE has not yet been identified. The critical importance of PPARα in regulating the enzymes involved in β-oxidation was established using mice in which the PPARα gene was inactivated by homologous recombination (17). In mutant animals the enzymes of the peroxisomal β-oxidation pathway are not induced by peroxisome proliferators (17).

The gene encoding the enzyme acyl-CoA synthetase is regulated by the alternative usage of three promoters and contains a functional PPRE in the C promoter (101). This enzyme is localized in peroxisomes, mitochondria, and microsomes, suggesting a role for PPAR in the regulation of extra-peroxisomal genes. Furthermore, there are two distinct functional PPREs in the promoter of the CYP4A6 gene, which encodes the microsomal cytochrome P450 fatty acid ϖ-hydroxylase (102). The same gene contains a cryptic PPRE that is activated by PPAR/RXR through relief of transcriptional repression by apolipoprotein regulatory protein 1 (ARP-1), another orphan member of the nuclear receptor superfamily (94). PPAR also controls the transcription of the mitochondrial HMG-CoA synthase gene by fatty acids and fibrates and does so via a PPRE located in the proximal promoter region (103). PPAR also binds to a pleiotropic element, called nuclear receptor regulatory element 1 (NRRE-1) in the regulatory sequences of the mitochondrial medium-chain acyl-CoA dehydrogenase gene, thereby regulating the transcriptional activity of this gene (95). Medium-chain acyl-CoA dehydrogenase is one of four chain length-specific mitochondrial enzymes that catalyze the initial reaction in mitochondrial β-oxidation. Surprisingly, the pleiotropic element NRRE-1 consists of four hexamers arranged as [← 1 $(n)_8$ 2 → $(n)_0$ 3 → $(n)_4$ ← 4], and hence does not contain a DR-1. This fact plus the finding that this element confers transcriptional regulation by the heterodimer RAR/RXR (104), by HNF-4 (105), and by COUP-TF (106) led the authors to propose that the specific structure of NRRE-1 permits competitive and cooperative interaction between various receptors, providing tissue-specific, hormone- and fatty acyl metabolite-sensitive modulation of medium-chain acyl-CoA dehydrogenase gene expression (95).

Various cytosolic proteins are induced upon administration of fatty acids and peroxisome proliferators. Two FA-binding proteins, liver FABP (107, 108) and adipose tissue-specific aP2 (13), contain functional PPRE-like sequences in the promoter regions of their genes. Furthermore, the gene encoding cytosolic malic enzyme is regulated by PPAR through a PPRE element (90), that is distinct from the previously identified TRE (109). A recent report described two distinct elements, PCK1

and PCK2, that bind the heterodimeric complex PPARγ2/RXRα in the regulatory region of the gene for PEPCK, an enzyme involved in gluconeo- and glyceroneogenesis. Only the latter element PCK2 was implicated in the transcriptional regulation of the PEPCK gene in adipose tissue (110).

PPAR not only plays a crucial role in intracellular lipid metabolism, but is also an important regulator of extracellular lipid metabolism. First, a PPRE was identified in the A site of the human apoA-I gene promoter. Through this PPRE, PPAR may counteract the repression of apoA-I gene transcription by fenofibrate. This phenomenon appears to be PPAR-independent (111). Second, PPAR is also involved in the control of human apoA-II gene expression (112). Third, transcription of the apoC-III gene is inhibited by fatty acids and peroxisome proliferators (113, 114) due to competition on the same RE between PPAR/RXR heterodimers and the liver-enriched transcriptional activator, HNF-4 (114). Finally, a PPRE sequence in the LPL promoter is capable of conferring PPAR transactivation to the lipoprotein lipase gene both in cells of hepatic origin and in adipocytes (115). These data indicate that PPARs also regulate the expression of secreted proteins and enzymes that regulate lipid transport and lipoprotein physiology.

Target gene activation and repression

Transactivation by nuclear receptors can occur through direct interaction with and stabilization of components of the transcription preinitiation complex, as has been demonstrated for COUP-TF, TR, and VDR, all of which bind directly to TFIIB (116–118). Alternatively, other factors, such as adaptors, coactivators and accessory proteins, may be required to transmit signals from the receptors to the core transcription machinery. Several coactivators interact with the nuclear receptor family members; for example TATA binding protein-Associated Factor$_{II}$ 30 (TAF$_{II}$30) (119), ER associated protein 160 (ERAP 160) (120), TR interacting protein 1 (Trip1) (121), and steroid receptor coactivator 1 (SRC-1) (122). Both components of the basic transcription machinery and the auxiliary bridging proteins transmit transactivation through interaction with the N-terminus (121) or the C-terminal ligand binding domain (119–121) of the receptor. In addition to these coactivators, two co-repressors for RAR and TR, termed nuclear receptor co-repressor (N-CoR) (123) and silencing mediator for retinoid and thyroid hormone receptors (SMRT) (124), have been identified. These corepressors interact with unliganded receptors and are released upon ligand binding, explaining the fact that TR and RAR repress transcription in absence of a ligand (123, 124). Whether similar mechanisms and similar coactivators and corepressors exist for the PPARs awaits further study.

In addition to corepressor-mediated inhibition of transcription by unliganded nuclear receptors, negative regulation also can occur through binding to negative REs. For example, specific REs bind the glucocorticoid receptor with high affinity but do not activate transcription, most likely due to an induced conformational change of the receptor (125, 126). Finally, *cis*-elements occupied by other *trans*-acting factors represent another way to exert negative transcriptional effects. Fibrates and fatty acids decrease the expression of several genes, such as those for apoC-III, acetyl-CoA carboxylase, and fatty acid synthase. Interestingly, the decrease in apoC-III transcription (113, 114) is linked to competition between the potent transcriptional activator HNF-4 and PPAR/RXR for the same RE (114). The involvement of PPARs in the negative responses of other genes to fibrates and fatty acids has not been established.

PPAR AND THE REGULATION OF EXTRACELLULAR LIPID METABOLISM

Effects of fibrates and fatty acids on the HDL constituents apoA-I and A-II

Increased levels of HDL are correlated with a decreased risk for coronary artery disease (127). The major proteins in HDL are apoA-I and A-II (127). The protective effect of HDL on atherosclerosis is correlated with the levels of specific HDL particles, those containing apoA-I (Lp A-I) and not those containing both apoA-I and A-II (Lp A-I:A-II) (128). Overexpression of human apoA-I in transgenic mice confers resistance to atherogenesis (129), whereas less protection is observed in mice overexpressing both human apoA-I and apoA-II (130). Furthermore, transgenic mice overexpressing mouse apoA-II exhibit increased atherosclerotic lesion development (131). Together, these data suggest that apoA-II is less protective than apoA-I in coronary heart disease.

In rodents, liver apoA-I and apoA-II mRNAs are inhibited by fenofibrate, dietary fish oils, and non-metabolizable fatty acids (87, 132). In humans, however, fibrates increase HDL levels and its major constituents, apoA-I and apoA-II albeit to a variable extent (133, 134). Fibrates primarily increase Lp A-I:A-II, although a small decrease may be observed in Lp A-I levels (135, 136). It is important to stress that important organism-specific differences exist between rodents and humans regarding HDL metabolism and its modification by peroxisome proliferators. Hence care should be taken when extrapolating the observations in rodents to the human situation.

Fibrates and fatty acids exert a negative effect on apoA-I transcription (111) via sequences localized in the

proximal promoter region of the human apoA-I gene. However, the negative effect of fibrates and fatty acids on apoA-I transcription is counteracted by a positive regulatory site that is responsive to PPAR, is localized in the apoA-I promoter A site, and binds other nuclear receptors such as ARP-1 and RXR (137). The delicate interplay of these opposite mechanisms of apoA-I regulation will determine the overall effects of fibrates on apoA-I gene expression. It is tempting to speculate that some peroxisome proliferators may be more potent in activating the A-site and will increase apoA-I gene transcription, whereas others may act primarily on the negative site, causing no change or a decrease in apoA-I gene transcription.

In humans, apoA-II plasma concentrations increase after fibrate treatment. This stimulation is, at least in part, due to the induction of hepatic apoA-II synthesis because peroxisome proliferators increase apoA-II mRNA and protein in human hepatocytes. The increase in expression of the human apoA-II gene caused by fibrates and fatty acids is mediated by PPAR, with PPAR/RXR heterodimers binding to an imperfect DR-1 in the apoA-II J site. This element also binds several other orphan receptors, such as HNF-4, EAR-2, EAR-3, and ARP-1 (138, 139).

Through these mechanisms, fibrates favor the occurrence of the less protective HDL profile consisting of increased Lp A-I:A-II and decreased LpA-I particles (135). Plasma concentrations of apoA-I are determined mainly by the degradation rate of apoA-I; apoA-II levels, and are proportional to the rate of apoA-II production. Furthermore, the production rate of apoA-II appears to be an important factor in the distribution of apoA-I between HDL particles LpA-I and Lp A-I:Lp A-II (140); LpA-I levels are inversely correlated with apoA-II production. An increase of apoA-II production, as seen after fibrate and FA treatment, would therefore result in a shift of apoA-I from Lp A-I to Lp A-I:A-II, resulting in lower Lp A-I and higher Lp A-I:A-II levels. This finding plus the increase in apoA-II synthesis offers a mechanism to explain decreased Lp A-I and increased Lp A-I:A-II levels in humans after fibrate treatment. The opposite effects of fibrates on levels of apoA-I (increased) and Lp A-I (decreased) in the plasma could, therefore, be due to an increase in expression and synthesis of apoA-II caused by fibrates.

Effect of fibrates and fatty acids on the metabolism of triglyceride-rich lipoproteins

Extracellular effects: LPL and apoC-III. The hypotriglyceridemic action of fibrates and fatty acids is attributable to both an enhanced catabolism of plasma triglyceride-rich particles and inhibition of secretion of triglyceride-rich particles from the liver (**Fig. 4**). The extracellular effects of fibrates and fatty acids on TG metabolism appear to be mediated via changes in expression of lipoprotein lipase (LPL) and apoC-III, major determinants of plasma TG concentrations.

LPL hydrolyzes the TG moiety of chylomicrons and VLDL particles (141, 142). The released free fatty acids are either oxidized to generate ATP in muscle, stored in adipose tissue, or secreted in milk by the mammary gland. LPL, therefore, occupies a pivotal position in both lipoprotein and energy metabolism. In addition, LPL is a ligand for several lipoprotein receptors, suggesting that it may have a role in particle clearance (reviewed in refs. 140, 142). The importance of LPL in lipoprotein metabolism is illustrated by the severe hypertriglyceridemia in patients with familial LPL deficiency (reviewed in ref. 142). Subtle abnormalities in LPL activity may predispose individuals to alterations in lipoprotein metabolism such as familial combined hyperlipidemia (143). LPL activity also is positively correlated with HDL cholesterol, a protective factor against coronary atherosclerosis (144, 145).

In contrast to LPL, apoC-III appears to inhibit clearance of plasma triglycerides. In numerous clinical, epidemiological, and genetic studies, apoC-III levels are correlated with plasma triglyceride concentrations (146–154). In transgenic animals, overexpressing apoC-III or lacking a functional apoC-III gene, plasma triglyceride levels are proportional to plasma apoC-III concentrations, providing evidence for the causal relationship of increased apoC-III levels to hypertriglyceridemia (155–157). Metabolic studies in these animals suggest that the primary abnormality is an impaired clearance of TG-rich lipoproteins due to interference with apoE-mediated uptake of these particles by cellular receptors (157–159).

It is assumed that one of the major causes of the hypotriglyceridemic action of fibrates and fatty acids involves an increase in LPL activity (160–162; reviewed in refs. 163, 164). In rats, LPL mRNA in liver (165) and adipose tissue (166) can be increased by fibrates and fatty acids. Furthermore, in hepatocytes and in preadipocytes, fibrates and fatty acids stimulate the transcription of the LPL gene via a PPRE (115). Thus, activation of LPL transcription by fibrates and fatty acids via this PPRE may contribute to the increase in LPL activity observed after treatment with these compounds. In addition, apoC-III expression is substantially reduced at the transcriptional level by fibrates (113, 114), resulting in diminished secretion of apoC-III (113, 114). Consistent with these findings, turn-over studies in humans indicate that fibrates reduce the apoC-III synthetic rates. The reduction in circulating apoC-III levels in response to fibrates should enhance the LPL-mediated effects on lipoprotein metabolism leading to an increased catabo-

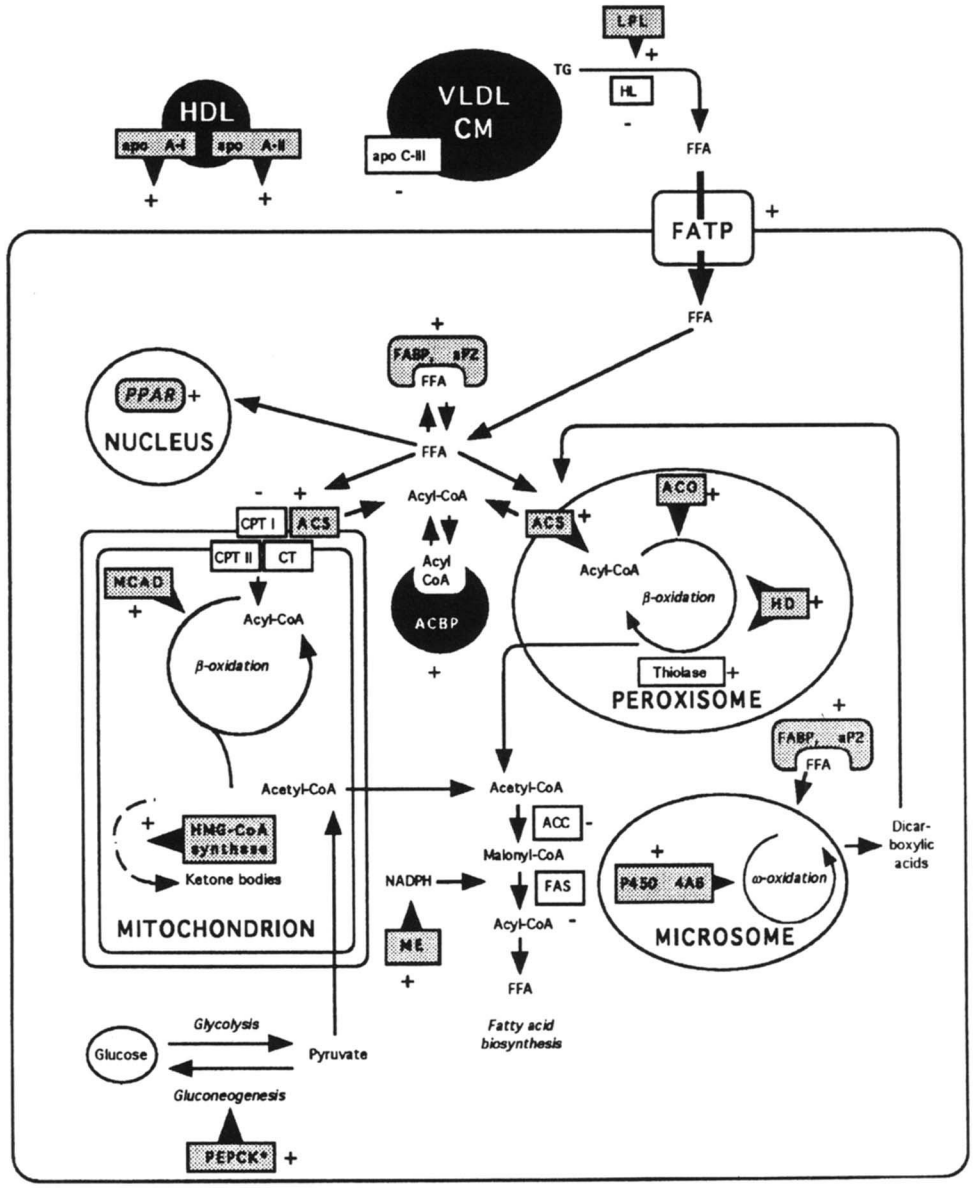

Fig. 4. Summary of the different genes regulated by PPARs and their roles in intra- and extracellular lipid metabolism. The genes encoding the proteins indicated in a grey box contain a functional PPRE. The proteins boxed with a white background are regulated by peroxisome proliferators, but no functional PPRE has been identified. The + or - signs indicate whether gene expression is stimulated or inhibited. Abbreviations can be found in the list of abbreviations. The concerted PPAR-mediated regulation of several genes involved in metabolism of TG-rich lipoproteins by fibrates and fatty acids explains the pleiotropic effects of fibrate therapy. In addition to the abbreviations used in Fig. 2, this figure contains the following abbreviations: ACBP, acyl-CoA binding protein; ACC, acetyl-CoA carboxylase; CM, chylomicron; CPT, carnitine palmitoyl transferase; CT, carnitine: acylcarnitine translocase; FAS, fatty acid synthase; FATP, fatty acid transport protein; FFA, free fatty acid.

lism of VLDL particles. Consequently, this dual action of fibrates on the expression of the LPL and apoC-III genes provides a potential model by which these drugs induce a reduction in plasma TG concentrations (reviewed in ref. 167). Furthermore, these fibrate-mediated effects on triglyceride metabolism should lower postprandial hypertriglyceridemia, a phenomenon favoring the development of atherosclerotic lesions. Together with the higher discussed changes in HDL metabolism, the reduction in postprandial hyperlipidemia should ultimately result in a less atherogenic lipoprotein profile.

Intracellular effects: fatty acid transport and metabolism, triglyceride synthesis. Fibrates exert their hypolipidemic

actions not only via enhanced catabolism of TG-rich lipoproteins, but also by concomitant increases in uptake and metabolism of the released fatty acids (168). The cellular uptake of long-chain fatty acids is facilitated by fatty acid transporters, such as the fatty acid transport protein (169). Preliminary studies indicate that the expression of fatty acid transporter protein in rat liver is enhanced by fibrate or fatty acid treatment (G. Martin, B. Staels, and J. Auwerx, unpublished results). After facilitated passage across the plasma membrane, acyl-CoA synthetase esterifies the fatty acids to acyl-CoA derivatives, thereby preventing efflux from the cell, rendering uptake unidirectional (169) and activating the fatty acid for further metabolism (170). Once activated, these acyl-CoA intermediates can be metabolized in the catabolic pathway of fatty acid β-oxidation or in anabolic pathways, by which fatty acids are converted into more complex lipids. Expression of the acyl-CoA synthetase gene and activity of the enzyme are stimulated by fibrates and fatty acids in a variety of tissues and cells (101, 171), an effect mediated by a PPRE located in the C promoter of the acyl-CoA synthetase gene. This PPAR-dependent induction of acyl-CoA synthetase gene expression by fibrates and fatty acids, together with the function of acyl-CoA synthetase in intracellular uptake and modification of fatty acids, suggests a positive feedback loop in which increases in acyl-CoA synthetase activity stimulate production of PPAR activators which, in turn, increase acyl-CoA synthetase activity, etc.

Peroxisome proliferator-induced acyl-CoA synthetase activity generates acyl-CoA esters that are used predominantly for β-oxidation (172, 173). Due to the pronounced increase of peroxisomal β-oxidation, associated with a more moderate induction of mitochondrial β-oxidation, after treatment with peroxisome proliferators, less acyl-CoA esters should be available to be utilized for TG synthesis. A reduction in acetyl-CoA carboxylase (174–176) and fatty acid synthase (175, 177) activities will inhibit de novo fatty acid synthesis, further diminishing the intracellular fatty acid levels available for TG synthesis (178). Moreover, peroxisome proliferators not only increase β-oxidation and decrease TG synthesis (178, 179), but also decrease apoB and VLDL production and secretion (176, 180–182). In summary, reduced secretion of VLDL particles and enhanced catabolism of TG-rich particles likely contribute to the hypolipidemic effect of fibrates.

Conclusions. The concerted PPAR-mediated regulation of several genes involved in metabolism of TG-rich lipoproteins leads to *1)* increased hydrolysis of TGs, *2)* stimulation of cellular fatty acid uptake and conversion to acyl-CoA derivatives, *3)* stimulation of the β-oxidation, and *4)* reductions in fatty acid and TG synthesis and VLDL production. All these effects act in concert to explain the hypolipidemic effect of fibrates and fatty acids in humans and rodents. In rodents, however, the relative contribution of the stimulated (peroxisomal) β-oxidation in lowering TG levels appears to be far more important than in humans. In fact, fibrate administration in rodents results in a sustained increase in peroxisomal β-oxidation associated with peroxisome proliferation, a phenomenon not observed in man.

PPARγ, A NUCLEAR RECEPTOR TRIGGERING ADIPOCYTE DIFFERENTIATION

White adipose tissue is composed of adipocytes, cells that play a central role in lipid homeostasis and the maintenance of energy balance in vertebrates. These cells store energy in the form of TGs during periods of nutritional abundance and release it in the form of fatty acids at times of nutritional deprivation. Too much white adipose tissue defines the obese state, whereas absence of white adipose tissue is associated with lipodystrophic syndromes. In contrast to the development of brown adipose tissue, which mainly takes place before birth, the development of white adipose tissue is the result of a differentiation/development process that continues throughout life (183). During development, pluripotent cells become increasingly restricted to specific differentiation pathways. Adipocyte differentiation from adipose precursor cells (adipoblasts) is orchestrated by two interdependently acting groups of transcription factors: PPARγ (13, 26) and the CCAATT enhancer binding protein (C/EBP) family of transcription factors (184–187). In contrast to the wide tissue distribution of the various C/EBPs, PPARγ shows an adipose-restricted pattern of expression. The currently favored hypothesis is that C/EBPβ induces the expression of PPARγ (187), which provides the initial trigger for the adipogenic program. Terminal differentiation requires then the concerted action of both PPARγ and C/EBPα (26). Consistent with these findings is the fact that PPAR activators, such as fibrates (188, 189) and fatty acids (26, 190, 191), induce adipocyte differentiation. In this context, it is interesting to note that prostanoids, which are potent inducers of adipose differentiation programs (192, 193), are the natural ligands of PPARγ (66, 67). Whether PPARγ is the only PPAR playing a role in the induction of adipocyte differentiation is unclear at present and awaits further study.

Several highly specialized proteins are induced during adipocyte differentiation, most of them involved in lipid storage and metabolism. Good examples of such proteins are aP2 (13), PEPCK (110), acyl-CoA synthetase (101, 171), and LPL (115). In each of these PPREs have been identified in the respective gene. The identification of a PPRE in the LPL promoter, an enzyme capable

of increasing the availability of fatty acids to the adipocyte, is highly interesting in this context. The observation that the LPL gene is more efficiently activated by PPARγ than by PPARα is consistent with a specific role for PPARγ in adipose tissue differentiation (26, 115). The increase in lipolytic capacity in the plasma that results from a PPARγ-mediated increase in LPL will result in an increased delivery of fatty acids to the cells. This increase in uptake should be further enhanced by the increase in acyl-CoA synthetase activity mediated by PPAR. Fatty acids are potent activators of PPAR (9, 14, 50) and will provide the necessary building blocks for TG accumulation, ultimately resulting in adipocyte differentiation. This hypothesis is supported by the observation that fatty acids and fatty acid analogues induce the expression of adipocyte-specific genes and enhance adipocyte conversion (26, 190, 191). This positive feedback loop, which involves PPARγ, LPL, acyl-CoA synthetase, fatty acid transport protein, and perhaps other genes, promotes and maintains the mature adipocyte phenotype.

FUTURE PERSPECTIVES

Since the cloning of the first PPAR in 1990 a wealth of information has accumulated on this group of receptors. These receptors modulate gene expression in response to environmental and dietary factors. PPARα and γ play a pivotal role in the control of metabolic functions in the hepatocyte and adipocyte, respectively. This has led to the speculation that dysfunction of PPARα might be associated with hyperlipoproteinemia whereas altered activity of PPARγ might be involved in the pathogenesis of obesity and non-insulin-dependent diabetes mellitus. Even less is known about the function of PPARδ.

In the near future, it is expected that we will be able to better understand the physiological role of PPARs through several parallel research strategies. *1)* Identification of ligands for the various PPAR types will provide researchers with powerful pharmacological tools to explore their physiology. A good example is the recent discovery of antidiabetic thiazolidinediones and prostaglandin J derivatives as specific ligands for PPARγ (66, 67). This discovery suggested a potential function of PPARγ in the pathogenesis of insulin resistance and non-insulin-dependent diabetes mellitus. Through intense screening efforts it is likely that ligands for PPARα and δ, still orphan receptors at present, will also be found. *2)* Targetted disruption of the PPAR genes by homologous recombination will be the second area of research that will help to elucidate the role of the various PPARs. In fact, disruption of the PPARα gene allowed researchers to unequivocally establish the role of this PPAR type in peroxisome proliferation (17). *3)* Finally, there is an urgent need for genetic studies to analyze the eventual involvement of PPARs in human diseases. When one takes the expression pattern of the PPARs into account, it is likely that PPARs must have important regulatory functions besides their roles in metabolic control.

We thank Delphine Cayet and Odile Vidal for excellent technical help in the research discussed in this manuscript. Jean-Charles Fruchart, Samir Deeb, Walter Wahli, Bruce Spiegelman, Guy Mannaerts, Paul Grimaldi, Senén Vilaró, and the members of the Laboratoire de Biologie des Régulations chez les Eucaryotes are acknowledged for stimulating discussions, support, suggestions, and for making data available before publication. Research reported in this work was supported by grants from INSERM, from the BioAvenir program, from the "Association de Recherche pour le Cancer (ARC 6403)", and from the "Fondation pour la Recherche Medicale." K. Schoonjans was supported by fellowships from ARC and IFN, J. Auwerx is a Research Director, and B. Staels is a Research Associate of the CNRS.

Manuscript received 27 October 1995, in revised form 10 November 1995, and in re-revised form 11 January 1996.

REFERENCES

1. Lock, E. A., A. M. Mitchell, and C. R. Elcombe. 1989. Biochemical mechanisms of induction of hepatic peroxisome proliferation. *Annu. Rev. Pharmacol. Toxicol.* **29:** 145–163.
2. Reddy, J. K., and N. D. Lalwani. 1983. Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* **12:** 1–58.
3. Osmundsen, H., J. Bremer, and J. I. Pedersen. 1991. Metabolic aspects of peroxisomal β-oxidation. *Biochim. Biophys. Acta.* **1085:** 141–158.
4. Bieri, F., and J. C. Lhuguenot. 1993. Toxicity of peroxisome proliferators. *Biochimie.* **75:** 263–268.
5. Osmundsen, H. 1982. Peroxisomal β-oxidation of long chain fatty acids: effects of high fat diets. *Ann. NY Acad. Sci.* **386:** 13–29.
6. Lalwani, N. D., K. Alvares, M. K. Reddy, M. N. Reddy, I. Parikh, and J. K. Reddy. 1987. Peroxisome proliferator-binding protein: identification and partial characterization of nafenopin-, clofibric acid-, and ciprofibrate-binding proteins from rat liver. *Proc. Natl. Acad. Sci. USA.* **84:** 5242–5246.
7. Isseman, I., and S. Green. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature.* **347:** 645–650.
8. Dreyer, C., G. Krey, H. Keller, F. Givel, G. Helftenbein, and W. Wahli. 1992. Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors. *Cell.* **68:** 879–887.
9. Gottlicher, M., E. Widmark, Q. Li, and J. A. Gustafsson. 1992. Fatty acids activate chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. *Proc.*

Natl. Acad. Sci. USA. **89:** 4653–4657.
10. Schmidt, A., N. Endo, S. J. Rutledge, R. Vogel, D. Shinar, and G. A. Rodan. 1992. Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. *Mol. Endocrinol.* **6:** 1634–1641.
11. Sher, T., H. F. Yi, W. McBride, and F. J. Gonzalez. 1993. cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. *Biochemistry.* **32:** 5598–5604.
12. Zhu, Y., K. Alvares, Q. Huang, M. S. Rao, and J. K. Reddy. 1993. Cloning of a new member of the peroxisome proliferator activated receptor gene family from mouse liver. *J. Biol. Chem.* **268:** 26817–26820.
13. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman. 1994. mPPARγ2: tissue-specific regulator of an adipocyte enhancer. *Genes & Dev.* **8:** 1224–1234.
14. Kliewer, S. A., B. M. Forman, B. Blumberg, E. S. Ong, U. Borgmeyer, D. J. Mangelsdorf, K. Umesono, and R. M. Evans. 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc. Natl. Acad. Sci. USA.* **91:** 7355–7359.
15. Amri, E-Z., F. Bonino, G. Ailhaud, N. A. Abumrad, and P. A. Grimaldi. 1995. Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. *J. Biol. Chem.* **270:** 2367–2371.
16. Greene, M. E., B. Blumberg, O. W. McBride, H. F. Yi, K. Kronquist, K. Kwan, L. Hsieh, G. Greene, and S. D. Nimer. 1995. Isolation of the human peroxisome proliferator activated receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expr.* **4:** 281–299.
17. Lee, S. S. T., T. Pineau, J. Drago, E. J. Lee, J. W. Owens, D. L. Kroetz, P. M. Fernandez-Salguero, H. Westphal, and F. J. Gonzalez. 1995. Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol. Cell. Biol.* **15:** 3012–3022.
18. Evans, R. M. 1988. The steroid and thyroid hormone receptor superfamily. *Science.* **240:** 889–895.
19. Tsai, M-J., and B. O'Malley. 1994. Molecular mechanisms of action of steroid/thydroid receptor superfamily members. *Annu. Rev. Biochem.* **63:** 451–486.
20. Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, E. Mark, P. Chambon, and R. M. Evans. 1995. The nuclear receptor superfamily: the second decade. *Cell.* **83:** 835–839.
21. Mangelsdorf, D. J., and R. M. Evans. 1995. The RXR heterodimers and orphan receptors. *Cell.* **83:** 841–850.
22. Chen, F., S. N. Law, and B. W. O'Malley. 1993. Identification of two mPPAR related receptors and evidence for the existence of five subfamily members. *Biochem. Biophys. Res. Commun.* **196:** 671–677.
23. Green, S., and W. Wahli. 1994. Peroxisome proliferator activated receptors: finding the orphan a home. *Mol. Cell Endocrinol.* **100:** 149–153.
24. Aperlo, C., P. Pognonec, R. Saladin, J. Auwerx, and K. Boulukos. 1995. Isolation and characterization of the hamster peroxisomal proliferator activated receptor hPPARγ, a member of the nuclear hormone receptor superfamily. *Gene.* **162:** 297–302.
25. Tontonoz, P., R. A. Graves, A. I. Budavari, H. Erdjument-Bromage, M. Lui, E. Hu, P. Tempst, and B. M. Spiegelman. 1994. Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPARγ and RXRα. *Nucleic Acids Res.* **22:** 5628–5634.
26. Tontonoz, P., E. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. *Cell.* **79:** 1147–1156.
27. Zhu, Y., C. Qi, J. R. Korenberg, X-N. Chen, D. Noya, M. S. Rao, and J. K. Reddy. 1995. Structural organization of mouse peroxisome proliferator activated receptor γ (mPPARγ) gene: alternative promoter use and different splicing yield two mPPARγ isoforms. *Proc. Natl. Acad. Sci. USA.* **92:** 7921–7925.
28. Gearing, K. L., A. Crickmore, and J. A. Gustafsson. 1994. Structure of the mouse peroxisome proliferator activated receptor α gene. *Biochem. Biophys. Res. Commun.* **199:** 255–263.
29. Leid, M., P. Kastner, and P. Chambon. 1992. Multiplicity generates diversity in the retinoic acid signaling pathways. *Trends Biochem. Sci.* **17:** 427–433.
30. Krey, G., H. Keller, A. Mahfoudi, J. Medin, K. Ozato, C. Dreyer, and W. Wahli. 1993. Xenopus peroxisome proliferator activated receptors: genomic organization, response element recognition, heterodimer formation with retinoid X receptor and activation by fatty acids. *J. Steroid Biochem. Mol. Biol.* **47:** 65–73.
31. Dreyer, C., H. Keller, A. Mahfoudi, V. Laudet, G. Krey, and W. Wahli. 1993. Positive regulation of peroxisomal β-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biol. Cell.* **77:** 67–77.
32. Apfel, R., D. Benbrook, E. Lernhardt, M. A. Ortiz, G. Salbert, and M. Pfahl. 1994. A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. *Mol. Cell. Biol.* **14:** 7025–7035.
33. Willy, P. J., K. Umesono, E. S. Ong, R. M. Evans, R. A. Heyman, and D. J. Mangelsdorf. 1995. LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes & Dev.* **9:** 1033–1045.
34. Luisi, B. F., W. X. Xu, Z. Otwinowski, L. P. Freedman, K. R. Yamamoto, and P. B. Sigler. 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. *Nature.* **352:** 497–505.
35. Schwabe, J. W., L. Chapman, J. T. Finch, and D. Rhodes. 1993. The crystal structure of the estrogen receptor DNA-binding domain bound to DNA; how receptors discriminate between their response elements. *Cell.* **75:** 567–578.
36. Green, S., and P. Chambon. 1988. Nuclear receptors enhance our understanding of transcriptional regulation. *Trends Genet.* **4:** 309–314.
37. Yu, R. T., M. McKeown, R. M. Evans, and K. Umesono. 1994. Relationship between *Drosophila* gap gene *tailles* and vertebrate nuclear receptor Tlx. *Nature.* **370:** 375–379.
38. Desvergne, B., and W. Wahli. 1994. PPAR: a key nuclear factor in nutrient/gene interactions. *In* Inducible Gene Expression. P. Bauerle, editor. Birkhauser, Boston. Vol 1: 142–176.
39. Forman, B. M., C. R. Yang, M. Au, J. Casanova, J. Ghysdael, and H. H. Samuels. 1989. A domain containing leucine-zipper-like motifs mediate novel in vivo interactions between the thyroid hormone and retinoic acid

receptors. *Mol. Endocrinol.* **3:** 1610–1626.
40. Barettino, D., M. M. Vivanco Ruiz, and H. G. Stunnenberg. 1994. Characterization of the ligand-dependent transactivation domain of the thyroid hormone receptor. *EMBO J.* **13:** 3039–3049.
41. Tora, L., J. White, C. Brou, D. Tasset, N. Webster, E. Scheer, and P. Chambon. 1989. The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell.* **59:** 477–487.
42. Nagpal, S., S. Friant, H. Nakshatri, and P. Chambon. 1993. RARs and RXRs: evidence for two autonomous transactivation functions (AF-1 and AF-2) and heterodimerization in vivo. *EMBO J.* **12:** 2349–2360.
43. Beekman, J. M., G. F. Allan, S. Y. Tsai, M-J. Tsai, and B. W. O'Malley. 1993. Transcriptional activation by the estrogen receptor. *Mol. Endocrinol.* **7:** 1266–1274.
44. Lees, J. A., S. E. Fawell, and M. G. Parker. 1989. Identification of two transactivation domains in the mouse oestrogen receptor. *Nucleic Acids Res.* **17:** 5477–5488.
45. Allan, G. F., X. H. Leng, S. Y. Tsai, N. L. Weigel, D. P. Edwards, M-J. Tsai, and B. W. O'Malley. 1992. Hormone and antihormone induce distinct conformational changes which are central to steroid receptor activation. *J. Biol. Chem.* **267:** 19513–19520.
46. Kurokawa, R., J. DiRenzo, M. Boehm, J. Sugarman, B. Gloss, M. G. Rosenfeld, R. A. Heyman, and C. K. Glass. 1994. Regulation of retinoid signalling by receptor polarity and allosteric control of ligand binding. *Nature.* **371:** 528–531.
47. Forman, B. M., K. Umesono, J. Chen, and R. Evans. 1995. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell.* **81:** 541–550.
48. Beck, F., S. Plummer, P. V. Senior, S. Byrne, S. Green, and W. J. Brammar. 1992. The ontogeny of peroxisome-proliferator-activated receptor gene expression in the mouse and rat. *Proc. R. Soc. Lond. [Biol]* **247:** 83–87.
49. Braissant, O., F. Foufelle, C. Scotto, M. Dauca, and W. Wahli. 1996. Differential expression of peroxisome proliferator-activated receptors: tissue distribution of PPARα, β and γ in the adult rat. *Endocrinology.* **137:** 354–366.
50. Keller, H., C. Dreyer, J. Medin, A. Mahfoudi, K. Ozato, and W. Wahli. 1993. Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA.* **90:** 2160–2164.
51. Gebel, T., M. Arand, and F. Oesch. 1992. Induction of the peroxisome proliferator activated receptor by fenofibrate in rat liver. *FEBS Lett.* **309:** 37–40.
52. Lemberger, T., B. Staels, R. Saladin, B. Desvergne, J. Auwerx, and W. Wahli. 1994. Regulation of the peroxisome proliferator-activated receptor alpha gene by glucocorticoids. *J. Biol. Chem.* **269:** 24527–24530.
53. Steineger, H. H., H. N. Sorensen, J. D. Tugwood, S. Skrede, O. Spydevold, and K. M. Gautvik. 1994. Dexamethasone and insulin demonstrate marked and opposite regulation of the steady-state mRNA level of the peroxisomal proliferator-activated receptor (PPAR) in hepatic cells. Hormonal modulation of fatty acid-induced transcription. *Eur. J. Biochem.* **225:** 967–974.
54. Lemberger, T., R. Saladin, M. Vásquez, F. Assimacopoulos, B. Staels, B. Desvergne, W. Wahli, and J. Auwerx. 1996. Expression of the peroxisome proliferator-activated receptor α gene is stimulated by stress and follows a diurnal rhythm. *J. Biol. Chem.* **271:** 1764–1769.
55. Neat, C. E., S. Thomassen, and H. Osmundsen. 1980. Induction of peroxisomal β-oxidation in rat liver by high fat diet. *Biochem. J.* **186:** 369–371.
56. Flatmark, T., A. Nilsson, J. Kvannes, T. S. Eikhom, M. H. Fukami, H. Kryvi, and E. N. Christiansen. 1988. On the induction of the enzyme systems for peroxisomal β-oxidation of fatty acids in rat livers by diets rich in partially hydrogenated fish oil. *Biochim. Biophys. Acta.* **962:** 122–130.
57. De Craemer, D., J. Vamecq, F. Roels, L. Vallee, M. Pauwels, and C. Van den Branden. 1994. Peroxisomes in liver, heart, and kidney of mice fed a commercial fish oil preparation: original data and review on peroxisomal changes induced by high fat diets. *J. Lipid Res.* **35:** 1241–1250.
58. Thomas, H., L. Schadt, M. Knehr, and F. Oesch. 1989. Effects of diabetes and starvation on the activity of raty liver epoxide hydrolases, gluthathione S-transferase and peroxisomal β-oxidation. *Biochem. Pharmacol.* **38:** 4291–4297.
59. Orellana, M., O. Fuentes, H. Rosenbluth, M. Lara, and V. E. 1992. Modulation of rat liver peroxisomal and microsomal fatty acid oxidation by starvation. *FEBS Lett.* **310:** 193–196.
60. Isseman, I., R. A. Prince, J. D. Tugwood, and S. Green. 1993. The peroxisome proliferator-activated receptor: retinoic X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. *J. Mol. Endocrinol.* **11:** 37–47.
61. Bronfman, M., L. Amigo, and M. N. Morales. 1986. Activation of hypolipidemic drugs to acyl-coenzyme A thioesters. *Biochem. J.* **239:** 781–784.
62. Raman, N., and C. DiRusso. 1995. Analysis of acyl-coenzyme A binding to the transcription factor FadR and identification of amino acid residues in the carboxyl terminus required for ligand binding. *J. Biol. Chem.* **270:** 1092–1097.
63. Hertz, R., I. Berman, and J. Bar-Tana. 1994. Transcriptional activation by amphipathic carboxylic peroxisomal proliferators is induced by the free acid rather than the acyl-CoA derivative. *Eur. J. Biochem.* **221:** 611–615.
64. Lehmann, J. M., L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ). *J. Biol. Chem.* **270:** 12953–12956.
65. Yu, K., W. Bayona, C. B. Kallen, H. P. Harding, C. P. Ravera, G. McMahon, M. Brown, and M. A. Lazar. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* **270:** 23975–23983.
66. Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans. 1995. 15-Deoxy-Δ12,14 prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. *Cell.* **83:** 803–812.
67. Kliewer, S. A., J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehman. 1995. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell.* **83:** 813–819.
68. Denner, L. A., N. L. Weigel, B. L. Maxwell, W. T. Schrader, and B. W. O'Malley. 1990. Regulation of progesterone receptor-mediated transcription by phosphorylation. *Science.* **250:** 1740–1743.

69. Aronica, S. M., and B. S. Katzenellenbogen. 1993. Stimulation of estrogen receptor-mediated transcription and alteration in the phosphorylation state of the rat uterine estrogen receptor by estrogen, cyclic adenosine monophosphate, and insulin-like growth factor-I. *Mol. Endocrinol.* **7:** 743–752.
70. Power, R. F., S. K. Mani, J. Codina, O. M. Conneely, and B. W. O'Malley. 1991. Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science.* **254:** 1636–1639.
71. Power, R. F., J. P. Lydon, O. M. Conneely, and B. W. O'Malley. 1991. Dopamine activation of an orphan of the steroid receptor superfamily. *Science.* **252:** 1546–1548.
72. Naar, A. M., J. M. Boutin, S. M. Lipkin, V. C. Yu, J. M. Holloway, C. K. Glass, and M. G. Rosenfeld. 1991. The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. *Cell.* **657:** 1267–1279.
73. Umesono, K., K. K. Murakami, C. C. Thompson, and R. M. Evans. 1991. Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and vitamin D3 receptors. *Cell.* **65:** 1255–1266.
74. Green, S. 1993. Promiscuous liaisons. *Nature.* **361:** 590–591.
75. Gronemeyer, H., and D. Moras. 1995. How to finger DNA. *Nature.* **375:** 190–191.
76. Wilson, T. E., T. J. Fahrner, M. Johnston, and J. Milbrandt. 1991. Identification of the DNA binding site for NGFI-B by genetic selection in yeast. *Science.* **252:** 1296–1300.
77. Ohno, C. K., H. Ueda, and M. Petkovich. 1994. The *Drosophila* nuclear receptors FTZ-F1α and FTZ-F1β compete as monomers for binding to a site in the *fushi tarazu* gene. *Mol. Cell. Biol.* **14:** 3166–3175.
78. Ueda, H., G-C. Sun, T. Murata, and S. Hirose. 1992. A novel DNA-binding motif abuts the zinc finger domain of insect nuclear hormone receptor FTZ-F1 and mouse embryonal long terminal repeat-binding protein. *Mol. Cell. Biol.* **12:** 5667–5672.
79. Carlberg, C., R. van Huijsduijnen, J. K. Staple, J. F. DeLamarter, and M. Becker-André. 1994. RZRs, a new family of retinoid-related orphan receptors that function as both monomers and homodimers. *Mol. Endocrinol.* **8:** 757–770.
80. Giguère, V., M. Tini, G. Flock, E. S. Ong, R. M. Evans, and G. Otulakowski. 1994. Isoforms-specific amino-terminal domains dictate DNA-bindings properties of RORα, a novel family of orphan nuclear receptors. *Genes & Dev.* **8:** 538–553.
81. Laudet, V., and G. Adelmant. 1995. Lonesome orphans. *Curr. Biol.* **5:** 124–127.
82. Dumas, B., H. P. Harding, H. S. Choi, K. A. Lehmann, M. Chung, M. A. Lazar, and D. D. Moore. 1994. A new orphan member of the nuclear hormone receptor superfamily closely related to Rev-Erb. *Mol. Endocrinol.* **8:** 996–1005.
83. Enmark, E., T. Kainu, M. Pelto-Huikko, and J. A. Gustafsson. 1994. Identification of a novel member of the nuclear receptor superfamily which is closely related to Rev-erbA. *Biochem. Biophys. Res. Commun.* **20:** 49–56.
84. Wilson, T. E., R. E. Paulsen, K. A. Padgett, and J. Milbrandt. 1992. Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. *Science.* **256:** 107–110.
85. Perlmann, T., and L. Jansson. 1995. A novel pathway for vitamin A signalling mediated by RXR heterodimerization with NGF1-B and NURR-1. *Genes & Dev.* **9:** 769–782.
86. Kliewer, S. A., K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans. 1992. Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature.* **358:** 771–774.
87. Berthou, L., R. Saladin, P. Yaqoob, P. Calder, J. C. Fruchart, P. Denefle, J. Auwerx, and B. Staels. 1995. Regulation of rat liver apolipoprotein A-I, apolipoprotein A-II, and acyl-CoA oxidase gene expression by fibrates and dietary fatty acids. *Eur. J. Biochem.* **232:** 179–187.
88. Bogazzi, F., L. D. Hudson, and V. M. Nikodem. 1994. A novel heterodimerization partner for thyroid hormone receptor. Peroxisome proliferator-activated receptor. *J. Biol. Chem.* **269:** 11683–11686.
89. Hertz, R., B. Kalderon, and J. Bar-Tana. 1993. Thyromimetic effect of peroxisome proliferators. *Biochimie.* **75:** 257–261.
90. Castelein, H., T. Gulick, P. E. Declercq, G. P. Mannaerts, D. D. Moore, and M. E. Baes. 1994. The peroxisome proliferator activated receptor regulates malic enzyme gene expression. *J. Biol. Chem.* **269:** 26754–26758.
91. Cooney, A. J., S. Y. Tsai, B. W. O'Malley, and M. J. Tsai. 1992. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) dimers bind to different GGTCA response elements, allowing COUP-TF to repress hormonal induction of the vitamin D_3, thyroid hormone, and retinoic acid receptors. *Mol. Cell. Biol.* **12:** 4153–4163.
92. Cooney, A. J., X. Leng, S. Y. Tsai, B. W. O'Malley, and M-J. Tsai. 1993. Multiple mechanisms of chicken ovalbumin upstream promoter transcription factor-dependent repression of transactivation by the vitamin-D, thyroid hormone, and retinoic acid receptors. *J. Biol. Chem.* **268:** 4152–4160.
93. Miyata, K., B. Zhang, S. L. Marcus, J. P. Capone, and R. A. Rachubinski. 1993. Chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds to a peroxisome-responsive element and antagonizes peroxisome proliferator-mediated signaling. *J. Biol. Chem.* **268:** 19169–19172.
94. Palmer, C. N. A., M-H. Hsu, A. S. Muerhoff, K. J. Griffin, and E. F. Johnson. 1994. Interaction of the peroxisome proliferator-activated receptor α with the retinoid X receptor α unmasks a cryptic peroxisome proliferator response element that overlaps an ARP-1-binding site in the CYP4A6 promoter. *J. Biol. Chem.* **269:** 18083–18089.
95. Gulick, T., S. Cresci, T. Caira, D. D. Moore, and D. P. Kelly. 1994. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc. Natl. Acad. Sci. USA.* **91:** 11012–11016.
96. Osumi, T., J. K. Wen, and T. Hashimoto. 1991. Two *cis*-acting regulatory elements in the peroxisome proliferator-responsive element enhancer region of rat acyl-CoA oxidase gene. *Biochem. Biophys. Res. Commun.* **175:** 866–871.
97. Tugwood, J. D., I. Isseman, R. G. Anderson, K. R. Bundell, W. L. McPheat, and S. Green. 1992. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5′ flanking sequence of the rat acyl-CoA oxidase gene. *EMBO J.* **11:** 433–439.
98. Zhang, B., S. L. Marcus, F. G. Sajjadi, K. Alvares, J. K.

Reddy, S. Subramani, R. A. Rachubinski, and J. P. Capone. 1992. Identification of a peroxisome proliferator-responsive element upstream of the gene encoding rat peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase. *Proc. Natl. Acad. Sci. USA.* **89:** 7541–7545.

99. Bardot, O., T. C. Aldridge, N. Latruffe, and S. Green. 1993. PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. *Biochem. Biophys. Res. Commun.* **192:** 37–45.
100. Zhang, B., S. L. Marcus, K. S. Miyata, S. Subramani, J. P. Capone, and R. A. Rachubinski. 1993. Characterization of protein-DNA interactions within the peroxisome proliferator-responsive element of the rat hydratase-dehydrogenase gene. *J. Biol. Chem.* **268:** 12939–12945.
101. Schoonjans, K., M. Watanabe, H. Suzuki, A. Mahfoudi, G. Krey, W. Wahli, P. Grimaldi, B. Staels, T. Yamamoto, and J. Auwerx. 1995. Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J. Biol. Chem.* **270:** 19269–19276.
102. Muerhoff, A. S., K. J. Griffin, and E. F. Johnson. 1992. The peroxisome proliferator activated receptor mediates the induction of CYP4A6, a cytochrome P450 fatty acid ω-hydroxylase, by clofibric acid. *J. Biol. Chem.* **267:** 19051–19053.
103. Rodriguez, J. C., G. Gil-Gomez, F. G. Hegardt, and D. Haro. 1994. Peroxisome proliferator activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. *J. Biol. Chem.* **269:** 18767–18772.
104. Raisher, B. R., T. Gulick, Z. Zhang, A. W. Strauss, D. D. Moore, and D. P. Kelly. 1992. Identification of novel retinoid-responsive element in the promoter region of the medium-chain acyl-coenzyme A dehydrogenase gene. *J. Biol. Chem.* **267:** 20264–20269.
105. Carter, M. E., T. Gulick, B. D. Raisher, T. Caira, J. A. A. Ladias, D. D. Moore, and D. P. Kelly. 1993. Hepatocyte nuclear factor-4 activates medium chain acyl-CoA dehydrogenase gene transcription by interacting with a complex regulatory element. *J. Biol. Chem.* **268:** 13805–13810.
106. Carter, M. E., T. Gulick, D. D. Moore, and D. P. Kelly. 1994. A pleiotropic element in the medium-chain acyl-coenzyme A dehydrogenase gene promoter mediates transcriptional regulation by multiple nuclear receptor transcription factors and defines novel receptor-DNA binding motifs. *Mol. Cell. Biol.* **14:** 4360–4372.
107. Isseman, I., R. Prince, J. Tugwood, and S. Green. 1992. A role for fatty acids and liver fatty acid binding protein in peroxisome proliferation. *Biochem. Soc. Trans.* **20:** 824–827.
108. Kaikaus, R. M., W. K. Chan, N. Lysenko, R. Ray, P. R. Ortiz de Montellano, and N. Bass. 1993. Induction of peroxisomal fatty acid β-oxidation and liver fatty acid-binding protein by peroxisome proliferators. Mediation via the cytochrome P-450IVA1 ω-hydroxylase pathway. *J. Biol. Chem.* **268:** 9593–9603.
109. Petty, K. J., B. Desvergne, T. Mitsuhashi, and V. M. Nikodem. 1990. Identification of a thyroid hormone response element in the malic enzyme gene. *J. Biol. Chem.* **265:** 7395–7400.
110. Tontonoz, P., E. Hu, J. Devine, E. G. Beale, and B. M. Spiegelman. 1995. PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol. Cell. Biol.* **15:** 351–357.
111. Vu-Dac, N., K. Schoonjans, B. Laine, J. C. Fruchart, J. Auwerx, and B. Staels. 1994. Negative regulation of the human apolipoprotein A-I promoter by fibrates can be attenuated by the interaction of the peroxisome proliferator-activated receptor with its response element. *J. Biol. Chem.* **269:** 31012–31018.
112. Vu-Dac, N., K. Schoonjans, V. Kosykh, J. Dallongeville, J-C. Fruchart, B. Staels, and J. Auwerx. 1995. Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. *J. Clin. Invest.* **96:** 741–750.
113. Staels, B., N. Vu-Dac, V. Kosykh, R. Saladin, J-C. Fruchart, J. Dallongeville, and J. Auwerx. 1995. Fibrates down-regulate apolipoprotein C-III expression independent of induction of peroxisomal acyl-Coenzyme A oxidase. *J. Clin. Invest.* **95:** 705–712.
114. Hertz, R., J. Bishara-Shieban, and J. Bar-Tana. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs, suppression of apolipoprotein C-III. *J. Biol. Chem.* **270:** 13470–13475.
115. Schoonjans, K., B. Staels, S. Deeb, and J. Auwerx. 1996. PPARα and PPARγ activators direct a tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. Submitted.
116. Ing, N. H., J. M. Beekman, S. Y. Tsai, M. J. Tsai, and B. W. O'Malley. 1992. Members of the steroid hormone receptor superfamily interact with TFIIB (S300-II). *J. Biol. Chem.* **267:** 17617–17623.
117. Blanco, J. C. G., I-M. Wang, S. Y. Tsai, M-J. Tsai, B. W. O'Malley, P. W. Jurutka, M. R. Haussler, and K. Ozato. 1995. Transcription factor TFIIB and the vitamin D receptor cooperatively activate ligand-dependent transcription. *Proc. Natl. Acad. Sci. USA.* **92:** 1535–1539.
118. Baniahmad, A., I. Ha, D. Reinberg, S. Y. Tsai, M. J. Tsai, and B. W. O'Malley. 1993. Interaction of human thyroid hormone receptor β with transcription factor TFIIB may mediate target gene derepression and activation by thyroid hormone. *Proc. Natl. Acad. Sci. USA.* **90:** 8832–8836.
119. Jacq, X., C. Brou, Y. Lutz, I. Davidson, P. Chambon, and L. Tora. 1994. Human TAFII-30 is present in a distinct TFIID complex and is required for transcriptional activation by the estrogen receptor. *Cell.* **79:** 107–117.
120. Halachmi, S., E. Marden, G. Martin, H. MacKay, C. Abbondanza, and M. Brown. 1994. Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. *Science.* **264:** 1455–1458.
121. Lee, J. W., F. Ryan, J. C. Swaffield, S. A. Johnston, and D. D. Moore. 1995. Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. *Nature.* **374:** 91–94.
122. Onate, S. A., S. Y. Tsai, M. J. Tsai, and B. W. O'Malley. 1995. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science.* **270:** 1354–1357.
123. Hörlein, A. J., A. M. Näär, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Söderström, C. K. Glass, and M. G. Rosenfeld. 1995. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature.* **377:** 397–404.
124. Chen, J. D., and R. M. Evans. 1995. A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature.* **377:** 454–457.
125. Sakai, D., S. Helms, J. Carlstedt-Duke, J. A. Gustafsson,

F. M. Rottman, and K. R. Yamamoto. 1988. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. *Genes & Dev.* **2:** 1144–1154.
126. Diamond, M. I., J. N. Miner, S. K. Yoshinaga, and K. R. Yamamoto. 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. *Science.* **249:** 1266–1272.
127. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
128. Puchois, P., A. Kandoussi, P. Fievet, J. L. Fourrier, M. Bertrand, E. Koren, and J. C. Fruchart. 1987. Apolipoprotein A-I-containing lipoproteins in coronary artery disease. *Atherosclerosis.* **68:** 35–40.
129. Rubin, E. M., R. M. Krauss, E. A. Spangler, J. G. Verstuyft, and S. M. Clift. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature.* **353:** 265–267.
130. Schultz, J. R., J. G. Verstuyft, E. L. Gong, A. V. Nichols, and E. M. Rubin. 1993. Protein composition determines the anti-atherogenic properties of HDL transgenic mice. *Nature.* **365:** 762–764.
131. Warden, C. H., C. C. Hedrick, J. H. Qiao, L. W. Castellani, and A. J. Lusis. 1993. Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science.* **261:** 469–472.
132. Staels, B., A. Van Tol, T. Andreu, and J. Auwerx. 1992. Fibrates influence the expression of genes involved in lipoprotein metabolism in a tissue-selective manner in the rat. *Arterioscler. Thromb.* **12:** 286–294.
133. Malmendier, C. L., and C. Delcroix. 1985. Effects of fenofibrate on high and low density lipoprotein metabolism in heterozygous familial hypercholesterolemia. *Atherosclerosis.* **55:** 161–169.
134. Mellies, M. J., E. A. Stein, P. Khoury, G. Lamkin, and C. J. Glueck. 1987. Effects of fenofibrate on lipids, lipoproteins and apolipoproteins in 33 subjects with primary hypercholesterolaemia. *Atherosclerosis.* **63:** 57–64.
135. Lussier-Cacan, S., J-M. Bard, L. Boulet, A. C. Nestruck, A-M. Grothé, J-C. Fruchart, and J. Davignon. 1989. Lipoprotein composition changes induced by fenofibrate in dysbetalipoproteinemia type III. *Atherosclerosis.* **78:** 167–182.
136. Bard, J. M., H. J. Parra, R. Camare, G. Luc, O. Ziegler, C. Dachet, E. Bruckert, P. Douste-Blazy, P. Drouin, B. Jacotot, J. L. De Gennes, U. Keller, and J. C. Fruchart. 1992. A multicenter comparison of the effects of simvastatin and fenofibrate therapy in severe primary hypercholesterolemia, with particular emphasis on lipoproteins defined by their apolipoprotein composition. *Metabolism.* **41:** 498–503.
137. Widom, R. L., J. A. Ladias, S. Kouidou, and S. K. Karathanasis. 1991. Synergistic interactions between transcription factors control expression of the apolipoprotein A-I gene in liver cells. *Mol. Cell. Biol.* **11:** 677–687.
138. Ladias, J. A. A., M. Hadzopoulou-Cladaras, D. Kardassis, P. Cardot, J. Cheng, V. Zannis, and C. Cladaras. 1992. Transcriptional regulation of human apolipoprotein genes apoB, apoCIII, and apoAII by members of the steroid hormone receptor superfamily HNF-4, ARP-1, EAR-2, and EAR-3. *J. Biol. Chem.* **267:** 15849–15860.
139. Cardot, P., J. Chambaz, D. Kardassis, C. Cladaras, and V. I. Zannis. 1993. Factors participating in the liver-specific expression of the human apolipoprotein A-II gene and their significance for transcription. *Biochemistry.* **32:** 9080–9093.
140. Ikewaki, K., L. A. Zech, M. Kindt, H. B. J. Brewer, and D. J. Rader. 1995. Apolipoprotein A-II production rate is a major factor regulating the distribution of apolipoprotein A-I among HDL subclasses LpA-I and LpA-I:A-II in normolipidemic humans. *Arterioscler. Thromb. Vasc. Biol.* **15:** 306–312.
141. Olivecrona, T., and G. Bengtsson-Olivecrona. 1987. Lipoprotein lipase from milk: the model enzyme in lipoprotein lipase research. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener Press, Chicago, IL. 15–58.
142. Auwerx, J., P. Leroy, and K. Schoonjans. 1992. Lipoprotein lipase: recent contributions from molecular biology. *Crit. Rev. Clin. Lab. Sci.* **29:** 243–268.
143. Babirak, S. P., B. S. Brown, and J. D. Brunzell. 1992. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arterioscler. Thromb.* **12:** 1176–1183.
144. Tsutsumi, K., Y. Inoue, A. Shima, K. Iwasaki, M. Kawamura, and T. Murase. 1993. The novel compound NO-1886 increases lipoprotein lipase activity with resulting elevation of high density lipoprotein cholesterol, and long-term administration inhibits atherogenesis in coronary arteries of rats with experimental atherosclerosis. *J. Clin. Invest.* **92:** 411–417.
145. Liu, M. S., F. R. Jirik, R. C. LeBoeuf, H. Henderson, L. W. Castellani, A. J. Lusis, Y. Ma, I. J. Forsythe, H. Zhang, E. Kirk, J. D. Brunzell, and M. R. Hayden. 1994. Alteration of lipid profiles in plasma of transgenic mice expressing human lipoprotein lipase. *J. Biol. Chem.* **269:** 11417–11424.
146. Wang, C-S., W. J. McConathy, H. U. Kloer, and P. Alaupovic. 1985. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J. Clin. Invest.* **75:** 384–390.
147. Schonfeld, G., P. I. Georges, J. Miller, P. Reilly, and J. Witztum. 1979. Apolipoprotein C-II and C-III levels in hyperlipoproteinemia. *Metabolism.* **28:** 1001–1009.
148. Stocks, J., G. Holdsworth, and D. J. Galton. 1979. Hypertriglyceridaemia associated with an abnormal triglyceride-rich lipoprotein carrying excess apolipoprotein C-III. *Lancet.* **ii:** 667–671.
149. Le, N-A., J. C. Gibson, and H. N. Ginsberg. 1988. Independent regulation of plasma apolipoprotein C-II and C-III concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins. *J. Lipid Res.* **29:** 669–677.
150. Malmendier, C. L., J-F. Lontie, C. Delcroix, D. Y. Dubois, T. Magot, and L. De Roy. 1989. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic patients. Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration. *Atherosclerosis.* **77:** 139–149.
151. Ginsberg, H. N., N-A. Le, I. J. Goldberg, J. C. Gibson, A. Rubinstein, P. Wang-Iverson, R. Norum, and W. V. Brown. 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI: evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J. Clin. Invest.* **78:** 1287–1295.
152. Rees, A., C. C. Shoulders, J. Stocks, D. J. Galton, and F. E. Baralle. 1983. DNA polymorphism adjacent to human apoprotein A-I gene: relation to hypertriglyceridemia. *Lancet.* **i:** 444–446.
153. Dammerman, M., L. A. Sandkuijl, J. L. Halaas, W.

Chung, and J. L. Breslow. 1993. An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3′ untranslated region polymorphisms. *Proc. Natl. Acad. Sci. USA.* **90:** 4562–4566.
154. Quarfordt, S. H., G. Michalopoulos, and B. Schirmer. 1982. The effect of human C apolipoproteins on the in vitro hepatic metabolism of triglyceride emulsions in the rat. *J. Biol. Chem.* **257:** 14642–14647.
155. Ito, Y., N. Azrolan, A. O'Connell, A. Walsh, and J. L. Breslow. 1990. Hypertriglyceridemia as a result of human apoC-III gene expression in transgenic mice. *Science.* **249:** 790–793.
156. Maeda, N., H. Li, D. Lee, P. Oliver, S. H. Quarfordt, and J. Osada. 1994. Targetted disruption of the apolipoprotein C-III gene in mice results in hypertriglyceridemia and protection from postprandial hypertriglyceridemia. *J. Biol. Chem.* **269:** 23610–23616.
157. de Silva, H. V., S. J. Lauer, J. Wang, W. S. Simonet, K. H. Weisgraber, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of human apolipoprotein C-III in transgenic mice results in an accumulation of apolipoprotein B48 remnants that is corrected by excess apolipoprotein E. *J. Biol. Chem.* **269:** 2324–2335.
158. Kowal, R. C., J. Herz, K. H. Weisgraber, R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1990. Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. *J. Biol. Chem.* **265:** 10771–10779.
159. Aalto-Setälä, K., E. A. Fisher, X. Chen, T. Chajek-Shaul, T. Hayek, R. Zechner, A. Walsh, R. Ramakrishnan, H. N. Ginsberg, and J. L. Breslow. 1992. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo-CIII and reduced apoE on the particles. *J. Clin. Invest.* **90:** 1889–1900.
160. Heller, F., and C. Harvengt. 1983. Effects of clofibrate, bezafibrate, fenofibrate, and probucol on plasma lipolytic enzymes in normolipidaemic subjects. *Eur. J. Clin. Pharmacol.* **23:** 57–63.
161. Sommariva, D., D. Bonfiglioloi, I. Pogliaghi, L. Zanaboni, and E. Balboni. 1983. Effects of procetofen on serum lipids and lipoproteins and on post-heparin lipase activities in type II and in type IV hyperlipoproteinemic patients. *Curr. Ther. Res.* **34:** 907–915.
162. Levy, E., L. Thibault, J. Turgeon, C. C. Roy, C. Gurbindo, G. Lepage, M. Godard, G. E. Rivard, and E. Seidman. 1993. Beneficial effect of fish-oil supplements on lipids, lipoproteins, and lipoprotein lipase in patients with glycogen storage disease type I. *Am. J. Clin. Nutr.* **57:** 922–929.
163. Tikkanen, M. J. 1992. Fibric acid derivatives. *Curr. Opin. Lipidol.* **3:** 29–33.
164. Larsen, M. L., and D. R. Illingworth. 1993. Triglyceride-lowering agents: fibrates and nicotinic acid. *Curr. Opin. Lipidol.* **4:** 34–40.
165. Staels, B., and J. Auwerx. 1992. Perturbation of developmental gene expression in rat liver by fibric acid derivatives: lipoprotein lipase and alpha-fetoprotein as models. *Development.* **115:** 1035–1043.
166. Murphy, M. C., A. Zampelas, S. M. Puddicombe, N. P. Furlonger, L. M. Morgan, and C. M. Williams. 1993. Pretranslational regulation of the expression of the lipoprotein lipase gene by dietary fatty acids in the rat. *Br. J. Nutr.* **70:** 727–736.
167. Auwerx, J., K. Schoonjans, J. C. Fruchart, and B. Staels. 1995. Transcriptional control of triglyceride metabolism; fibrates change the expression of the LPL and apoC-III genes by activating the nuclear receptor PPAR. *Atherosclerosis.* In press.
168. Frenkel, B., J. Bishara-Shieban, and J. Bar-Tana. 1994. The effect of beta, beta′-tetramethylhexadecanedioic acid (MEDICA 16) on plasma very-low-density lipoprotein metabolism in rats: role of apolipoprotein C-III. *Biochem. J.* **298:** 409–414.
169. Schaffer, J. E., and H. F. Lodish. 1994. Expression cloning and characterization of a novel long chain fatty acid transport protein. *Cell.* **79:** 427–436.
170. Suzuki, H., Y. Kawarabayasi, J. Kondo, T. Abe, K. Nishikawa, S. Kimura, T. Hashimoto, and T. Yamamoto. 1990. Structure and regulation of rat long-chain acyl-CoA synthetase. *J. Biol. Chem.* **265:** 8681–8685.
171. Schoonjans, K., B. Staels, P. Grimaldi, and J. Auwerx. 1993. Acyl-CoA synthetase mRNA expression is controlled by fibric-acid derivatives, feeding and liver proliferation. *Eur. J. Biochem.* **216:** 615–622.
172. Aarsland, A., and R. Berge. 1990. Peroxisome proliferating sulphur- and oxy-substituted fatty acid analogues are activated to acyl coenzyme A thioesters. *Biochem. Pharmacol.* **41:** 53–61.
173. Skrede, S., M. Narce, S. Bergseth, and J. Bremer. 1989. The effects of alkylthioacetic acids (3-thia fatty acids) on fatty acid metabolism in isolated hepatocytes. *Biochim. Biophys. Acta.* **1005:** 296–302.
174. Maragandakis, M. E., and H. Hankin. 1971. On the mode of action of lipid lowering agents. V. Kinetics of the inhibition in vitro of rat acetyl-CoA carboxylase. *J. Biol. Chem.* **246:** 348–354.
175. Toussant, N. J., M. D. Wilson, and S. D. Clarke. 1981. Coordinate suppression of liver acetyl CoA carboxykinase and fatty acid synthase by polyunsaturated fat. *J. Nutr.* **111:** 146–163.
176. Asiedu, D. K., A. Al-Shurbaji, A. C. Rustan, I. Björkhem, and R. K. Berge. 1995. Hepatic fatty acid metabolism as a determinant of plasma and liver triacylglycerol levels. Studies on tetradecylthioacetic and tetradecylthiopropionic acids. *Eur. J. Biochem.* **227:** 715–722.
177. Blake, W. L., and S. D. Clarke. 1990. Suppression of rat hepatic fatty acid synthase and S14 transcription by dietary polyunsaturated fat. *J. Nutr.* **120:** 1727–1729.
178. Rustan, A. C., E. N. Christiansen, and C. A. Drevor. 1992. Serum lipids, hepatic glycerolipid metabolism and peroxisomal fatty acid oxidation in rats fed omega-3 and omega-6 fatty acids. *Biochem. J.* **283:** 333–339.
179. Kalderon, B., R. Hertz, and J. Bar-Tana. 1992. Tissue selective modulation of redox and phosphate potentials by beta, beta′-methyl-substituted hexadecanedioic acid. *Endocrinology.* **131:** 1629–1635.
180. Skrede, S., J. Bremer, R. K. Berge, and A. C. Rustan. 1994. Stimulation of fatty acid oxidation by a 3-thia fatty acid reduces triacylglycerol secretion in cultured rat hepatocytes. *J. Lipid Res.* **35:** 1395–1404.
181. Bar-Tana, J., G. Rose-Kahn, B. Frenkel, Z. Shafer, and M. Fainaru. 1988. Hypolipidaemic effect of beta, beta′-methyl-substituted hexadecanedioic acid (MEDICA 16) in normal and nephrotic rats. *J. Lipid Res.* **29:** 431–441.
182. Lamb, R. G., J. C. Koch, and S. R. Bush. 1993. An enzymatic explanation of the differential effects of oleate and gemfibrozil on cultured hepatocyte triacylglycerol and phosphatidylcholine biosynthesis and secretion. *Bio-*

chim. Biophys. Acta. **1165:** 299–305.
183. Flier, J. S. 1995. The adipocyte: storage depot or node on the energy information superhighway. *Cell.* **80:** 15–18.
184. Christy, R. J., V. W. Yang, J. M. Ntambi, D. E. Geiman, W. H. Landschulz, A. D. Friedman, Y. Nakabeppu, T. J. Kelly, and M. D. Lane. 1989. Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. *Genes & Dev.* **3:** 1323–1335.
185. Freytag, S. O., and T. J. Geddes. 1992. Reciprocal regulation of adipogenesis by Myc and C/EBPα. *Science.* **256:** 379–382.
186. Freytag, S. O., D. L. Paielli, and J. D. Gilbert. 1994. Ectopic expression of the CCAAT/enhancer-binding protein α promotes the adipogenic program in a variety of mouse fibroblastic cells. *Genes & Dev.* **8:** 1654–1663.
187. Wu, Z., Y. Xie, N. L. R. Bucher, and S. R. Farmer. 1995. Conditional ectopic expression of C/EBPβ in NIH-3T3 cells induces PPARγ and stimulates adipogenesis. *Genes & Dev.* **9:** 2350–2363.
188. Brandes, R., R. Hertz, R. Arad, S. Naishtat, S. Weil, and J. Bar-Tana. 1987. Adipocyte conversion of cultured 3T3-L1 preadipocytes by bezafibrate. *Life Sci.* **40:** 935–941.
189. Gharbi-Chibi, J., M. Teboul, J. Bismuth, J. Bonne, and J. Torresani. 1993. Increase of adipose differentiation by hypolipidemic fibrate drugs in ob 17 preadipocytes: requirement for thyroid hormones. *Biochim. Biophys. Acta.* **1177:** 8–14.
190. Amri, E-Z., B. Bertrand, G. Ailhaud, and P. Grimaldi. 1991. Regulation of adipose cell differentiation. I. Fatty acids are inducers of the aP2 gene expression. *J. Lipid Res.* **32:** 1449–1456.
191. Chawla, A., and M. A. Lazar. 1994. Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival. *Proc. Natl. Acad. Sci. USA.* **91:** 1786–1790.
192. Negrel, R., D. Gaillard, and G. Ailhaud. 1989. Prostacyclin as a potent effector of adipose-cell differentiation. *Biochem. J.* **257:** 399–405.
193. Gaillard, D., R. Negrel, M. Lagarde, and G. Ailhaud. 1989. Requirement and role of arachidonic acid in the differentiation of pre-adipose cells. *Biochem. J.* **257:** 389–397.