CMLS, Cell. Mol. Life Sci. 59 (2002) 790–798
1420-682X/02/050790-09 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

PPARs: transcriptional effectors of fatty acids and their derivatives

A. K. Hihi, L. Michalik and W. Wahli***

Institut de Biologie Animale, Bâtiment de Biologie, Université de Lausanne, 1015 Lausanne (Switzerland),
Fax + 41 21 692 41 15, e-mail: walter.wahli@iba.unil.ch

Abstract. Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that mediate the effects of fatty acids and their derivatives at the transcriptional level. These receptors stimulate transcription after activation by their cognate ligand and binding to the promoter of target genes. In this review, we discuss how fatty acids affect PPAR functions in the cell. We first describe the structural features of the ligand binding domains of PPARs, as defined by crystallographic analyses. We then present the ligand-binding characteristics of each of the three PPARs (α, β/δ, γ) and relate ligand activation to various cellular processes: (i) fatty acid catabolism and modulation of the inflammatory response for PPARα, (ii) embryo implantation, cell proliferation and apoptosis for PPARβ, and (iii) adipocytic differentiation, monocytic differentiation and cell cycle withdrawal for PPARγ. Finally, we present possible cross-talk between the PPAR pathway and different endocrine routes within the cell, including the thyroid hormone and retinoid pathways.

Key words. Peroxisome proliferator-activated receptor; nuclear receptors; transcription; ligand; eicosanoids; thiazolidinediones; fatty acid catabolism; differentiation.

Introduction

The understanding of tissue-specific effects of fatty acids relies on our knowledge of how these substances and their derivatives flux and signal in the organism. This includes how they are taken up, assimilated, transported in the blood, distributed to the cells and then either utilized as membrane constituents or metabolized to downstream products, some of them being very potent biological mediators. The activity of fatty acids and their metabolites is presently in the limelight. Alterations in their homeostasis are considered as key causes of some of the major pathologies of modern times, such as diabetes, obesity, cardiovascular diseases and cancer. Initially, the effects of fatty acids were thought to be mediated via changes in cellular membrane composition or via effects on signaling cascades [1]. The latter have been extensively studied recently, opening the new field of transcriptional regulation of gene activity via fatty acids [2]. It is through the discovery of soluble nuclear receptors for fatty acids that a direct link between fatty acids and gene regulation has been demonstrated. Indeed, it is now well established that as members of the steroid/thyroid hormone receptors family, the peroxisome proliferator-activated receptors (PPARs) [3] mediate the effects of fatty acids on gene expression and cell fate.

In this review, we summarize newly acquired knowledge regarding fatty acids as 'hormones' related to PPARs. First, we will discuss the structural properties of these receptors with respect to ligand binding. We will then describe the functional characteristics of each of the three PPAR isotypes in relation to ligand diversity and the effect on cellular processes. Possible cross-talk between PPARs and other lipid signaling routes will then be discussed, and finally we will conclude by presenting likely scenarios for the evolution of the field.

* Corresponding author.
** Present address: McGill University, Department of Biology, H3A1B1 Montreal (Canada)

Structural organization of PPARs

The PPARs belong to the superfamily of the steroid/thyroid nuclear hormone receptors [4, 5]. They have been identified in many species, such as *Xenopus* [6], mouse [7], rat [8] and human [9]. There are three isotypes (*α, β/δ, γ*; NR1C1, NR1C2 and NR1C3, respectively, according to the unified nomenclature of nuclear receptors [10]) discovered so far, which share the typical domain organization of nuclear receptors [11] (fig. 1A). The N-terminal A/B domain contains a ligand-independent transactivation function. In the *α* and *γ* isotypes, the activity of this domain can be regulated by mitogen-activated protein kinase (MAPK) phosphorylation [12, 13]. The C domain is the DNA binding domain with its typical two zinc-finger-like motifs, as previously described for the steroid receptors [14]. The E/F domain is the ligand binding domain. It contains a ligand-dependent transactivation function, AF-2 [6–8], and is able to interact with transcriptional coactivators such as SRC-1 [15–17] and CBP [18–20], in a ligand-dependent manner. PPAR acts on promoters of target genes as a heterodimer with its obligate partner RXR (NR2B) [10, 21–23], the nuclear receptor for 9-*cis* retinoic acid [24].

We herein focus on the characteristics of the ligand binding domain, for which interesting findings have emerged. For a comparative analysis of the three PPAR isotypes we can refer the readers to a comprehensive review on PPARs [25].

The ligand binding domain

The ligand binding domain (LBD) of PPARs contains a transactivation function that was first characterized in transfection assays, by using the PPAR LBD fused to the estrogen receptor DNA binding domain [6, 7]. These studies determined that compounds inducing peroxisome proliferation were able to activate PPARs. Further studies showed that PPARs could also be activated by a variety of polyunsaturated fatty acids, at concentrations in the micromolar range [8, 22], as well as by small synthetic molecules such as thiazolidinediones and L-tyrosine analogs in the nanomolar range [26]. The observed diversity of putative PPAR ligands led to the speculation that it was reflecting some unusual structural properties of the ligand binding domain. Nevertheless, the PPAR LBD presents an overall tridimensional fold, very similar to that of other receptors [27]. The PPAR*α* [28], PPAR*β/δ* [29] and PPAR*γ* LBD [30] (fig. 1B) consist of 13 *α* helices and a small four-stranded *β* sheet forming a large Y-shaped hy-

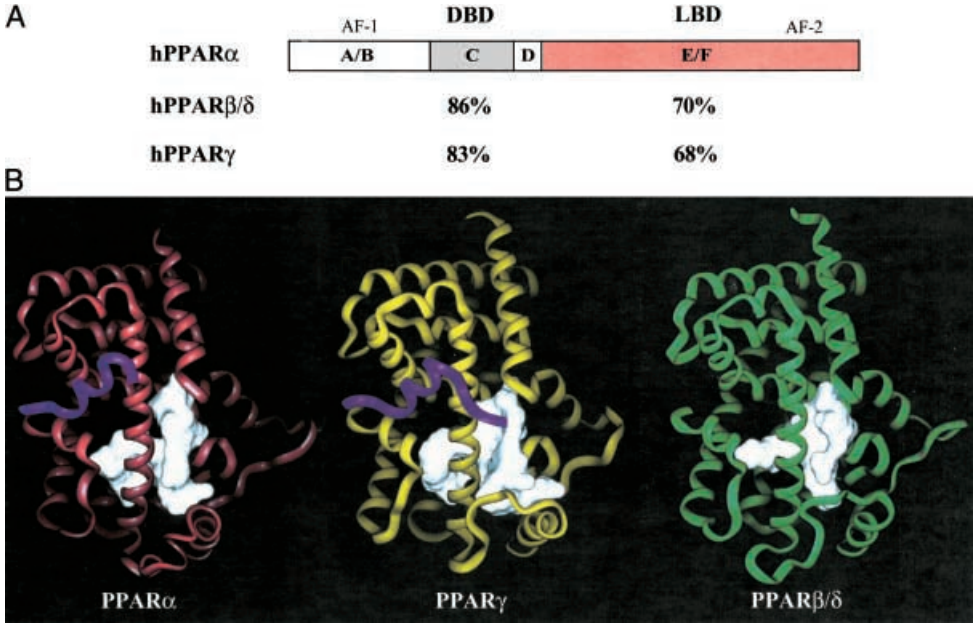

Figure 1. Structure of PPARs. (*A*) Schematic view of the domain organization of PPARs. Structural and functional domains are depicted. The A/B domain contains the activation function 1 (AF-1), which has a ligand-independent transcriptional activity. The C domain corresponds to the DNA binding domain (DBD). The E/F domain contains the ligand binding domain (LBD) and carries the activation function 2 (AF-2), which has a ligand-dependent transcriptional activity. The percentage of amino acid identity of the DBDs and LBDs of human PPAR*β* and PPAR*γ* compared with PPAR*α* is indicated. (*B*) Three-dimensional structure of ligand binding domains of PPARs. A comparison of the X-ray crystal structures of PPAR*α* (red worm), PPAR*γ* (yellow worm) and PPAR*β/δ* (green worm) is shown. Each PPAR is complexed to a high-affinity ligand (not pictured). PPAR*α* and PPAR*γ* are shown associated to LXXLL peptides (purple worms), the signature motif of the receptor coactivators. For each PPAR, the solvent-accessible ligand binding pocket is displayed as an off-white surface. (Reproduced from [28]).

drophobic pocket. This pocket represents the ligand binding cavity and has a volume of approximately 1300 Å^3, which is about twice that of the other nuclear receptors. For example, the ligand binding cavity of the thyroid hormone receptor has a volume of around 600 Å^3 [31]. In the case of PPARβ and -γ, the ligands occupy ~30–40% of the pocket, in contrast to the thyroid hormone receptor, where the ligand fills around 90% of the pocket [31]. Besides its large size, another characteristic feature of the PPAR ligand binding pocket is that its bottom portion is sealed by helix 2′, which is absent in other nuclear receptors. This particular helix may increase the size of the pocket, and possibly participates in an entry channel for the ligand. The position of the ligand in the PPARβ LBD was determined for eicosapentaenoic acid (EPA). The carboxylic acid of EPA interacts directly with the C-terminal helix containing the activation function 2 core, and its hydrophobic tail can adopt two conformations within the cavity, each of which is stabilized by hydrophobic interactions with the LBD. However, the pocket of PPARβ LBD is somewhat narrow at the vicinity of the AF-2 helix, which makes it hard to accommodate some ligands such as tyrosine-based molecules and contributes to the particular ligand binding profile of the β isotype [28]. Interestingly, the structural alignment of the ligand binding cavities of PPARα and PPARγ, combined with mutant analysis, showed that the ligand selectivity depends on the identity of a single amino acid in helix 5 (tyrosine in PPARα and histidine in PPARγ). This selectivity seems to be conserved between different ligand classes and corresponds to an intrinsic property of the receptors [28]. The characteristics of the PPAR LBDs give insight into the propensity of PPARs to interact with a variety of natural and synthetic compounds [29, 30].

PPAR activation and biological consequences

PPARα

Peroxisome proliferators (PPs) were known to affect peroxisomal β-oxidation of fatty acids, besides their effects on increasing peroxisome numbers in rodents. Since they were shown to be PPARα activators, the effects of PPARα on fatty acid catabolism were investigated. These analyses revealed that PPARα controls the transcriptional rate of genes encoding key enzymes of the fatty acid catabolism pathway (see fig. 2). Fatty acid oxidation takes place in specialized organelles: peroxisomes and mitochondria for β-oxidation, and microsomes for ω-oxidation. Importantly, PPARα transcriptionally regulates the production of enzymes such as acyl-coenzyme A (CoA) oxidase [6], the key enzyme in the peroxisomal β-oxidation pathway, carnitine palmitoyl transferase I [32], implicated in the translocation of fatty acids across the inner mitochondrial membrane, as well as CYP4A6, which is an important microsomal ω-hydroxylase [33].

Interestingly, fatty acids directly affect transcription by activating PPARα [8, 22, 34]. Moreover, it is possible to establish a hierarchy within the list of activators. The best natural PPARα agonists are long-chain polyunsaturated fatty acids such as arachidonic acid and linoleic acid, while the weakest ones are the short saturated fatty acids. These observations suggested that fatty acids might act as hormones that directly bind to the nuclear receptor PPARα. The rationale behind this diversity of PPARα activators, however, was not clear. To establish that some of these activators are true ligands, some indirect means were used. A first approach, called the CARLA method [16], was developed to exploit the interaction of nuclear receptors with transcriptional coactivator proteins such as SRC-1 upon ligand binding. This interaction between the receptor and the coactivator is mediated through a ligand-dependent rearrangement of the LBD of the receptor, involving particularly helix 12 [15, 16]. The analysis of the interaction profile between PPARα and SRC1 in the presence of a putative ligand provided results consistent with the transactivation profile observed earlier. In general, the polyunsaturated fatty acids were found to be better ligands than the saturated ones. The ligand-induced LBD conformational change was used in various other in vitro approaches. One of these methods allows the detection of ligands based on their ability to increase PPAR/RXR DNA binding in gel shift assays. This ligand-induced complex formation assay (LIC) provided results similar to CARLA [35, 36], as did an assay called differential protease sensitivity assay (DPSA), based on ligand-induced protease resistance of the LBD [37]. In addition, consistent results with respect to polyunsaturated fatty acid binding to PPARα were also obtained using a fluorescence-based method of ligand displacement [38]. Using this assay, polyunsaturated fatty acids such as arachidonic acid and linoleic acid showed apparent K_d values in the nanomolar range (17.3 nM for arachidonic acid, 4.8 nM for linoleic acid). Further investigations identified some eicosanoids as PPARα ligands. In particular, leukotriene B4 (LTB_4) produced from arachidonic acid via the lipoxygenase pathway, binds to PPARα with a K_d of 60–90 nM [38, 39]. The discovery that this molecule, which is well characterized for its implication in chemotaxis [40], is a PPARα ligand provided a link between this nuclear receptor and inflammation. Confirming this observation, the duration of a LTB_4 or arachidonic acid-induced inflammatory response is enhanced in the PPARα knockout mouse compared to the wild-type mouse. This result suggests that by binding to PPARα, LTB_4 might induce its own degradation via the ω- and β-oxidation pathways through PPARα activation. In PPARα knockout mice, these catabolic pathways would not be stimulated, hence the increased duration of the inflamma-

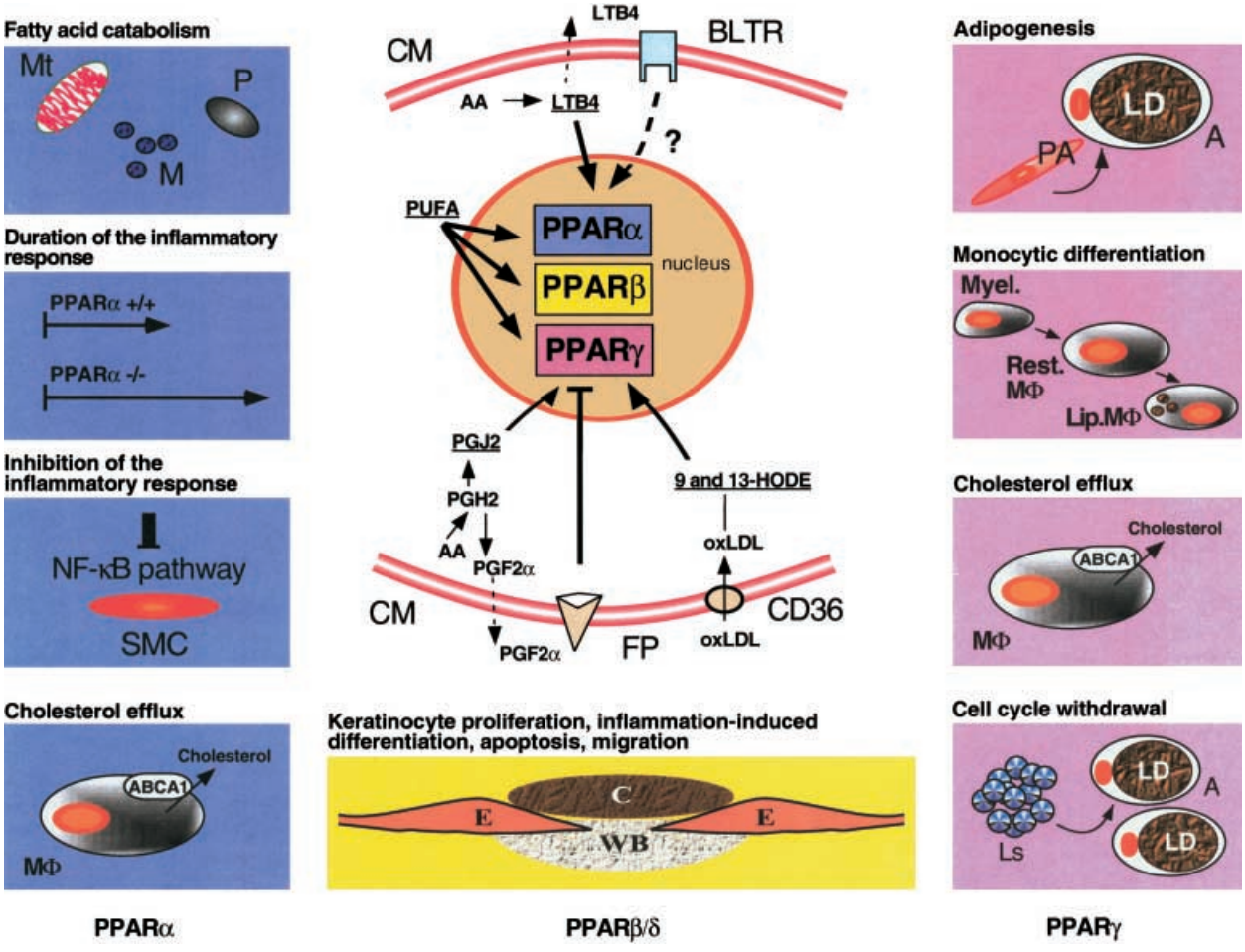

Figure 2. Schematic representation of PPAR functions. PPARs are located in the cell nucleus, where they modulate gene expression, mostly in a ligand-dependent manner. PPARα (blue boxes) is activated by polyunsaturated fatty acids (PUFA) and by an arachidonic acid (AA) derivative, the LTB_4. This latter also binds a membrane receptor (BLTR) with a very high affinity. The cross-talk between the membrane and the nuclear receptor for LTB4 is not yet elucidated. PPARα is able to enhance expression of genes involved in fatty acid catabolism in mitochondria (Mt), peroxisomes (P) and microsomes (M). In parallel, PPARα is capable of reducing the duration of an inflammatory reaction in wild-type mice as compared to PPARα –/– mice, of inhibiting the inflammatory response in smooth muscle cells (SMC) and can stimulate cholesterol efflux from human macrophages (MΦ). PPARβ (yellow boxes) is activated by PUFA. In an in vivo model of skin wound healing (E, epidermis; C, clot; WB, wound bed), PPARβ was recently shown to be involved in the control of keratinocyte proliferation, in inflammation-induced keratinocyte differentiation and in the increase of keratinocyte resistance to TNF-α-induced apoptosis. In addition, PPARβ is also implicated in embryo implantation, myelinization, lipid metabolism and adiposity. PPARγ (purple boxes) is activated by PUFA, by a PGJ_2 metabolite, which derives from AA and by oxidized LDL components, 9- and 13-HODE, which are internalized in the cell via the CD36 scavenger receptor. PPARγ is a key regulator of adipogenesis and is implicated in the differentiation of preadipocytes (PA) to adipocytes (A), characterized phenotypically by their lipid droplets (LD). Monocytic differentiation is also influenced by PPARγ. In the presence of PPARγ and RXR ligands, myeloid cell precursors (Myel.) become resting macrophages (Rest. MΦ). These resting MΦ can be turned to lipid-containing MΦ (lip. MΦ) when the PPARγ and PXR ligands are maintained. In human macrophages (MΦ), and in association with PPARα, PPARγ can stimulate cholesterol efflux. PPARγ is also capable of withdrawing liposarcoma-derived cells (Ls) from cell division to trigger their differentiation to adipocytes (A). Besides transcriptional activation, PPARγ function can be inhibited by PGF-2α, which binds to a membrane receptor (FP) located at the cell membrane (CM). This triggers MAPK activation, which phosphorylates PPARγ, and inhibits its function.

tory response (see fig. 2). Consistent with PPARα being involved in the control of inflammatory responses, cutaneous wound healing was shown to be impaired in PPARα null mice during the inflammatory phase of the process [41]. Along the same lines, PPARα ligands were shown to inhibit the inflammatory response in aortic smooth muscle cells [42], an effect that is lost in the PPARα null mice [43]. This occurs through the inhibition of interleukin-1-mediated expression of interleukin-6 and cyclooxygenase-2. The transcriptional inhibition of this key enzyme of the inflammation process occurs as a result of a PPARα-dependent repression of the nuclear factor kappa B (NF-κB) pathway (see fig. 2). In addition to LTB_4, another lipoxygenase metabolite, the 8(*S*)-hydroxyeicosatetraenoic acid (HETE), was also revealed as a PPARα ligand [16, 35]. This eicosanoid is associated

with phorbol ester-induced inflammation, but so far no study has addressed the question of a role of PPARα in this particular mechanism. In addition to its regulatory role in inflammation processes, and in association with PPARγ, PPARα was shown to mediate cholesterol efflux from human macrophages. This is likely due to the induction of the nuclear receptor LXR (NR1H3, [10]) expression, which in turn upregulates the expression of the reverse cholesterol transporter ABCA1. It will be interesting in the future to assess the clinical effects of PPARα agonists on acute inflammatory situations and atherosclerosis.

PPARβ

PPARβ is so far the most elusive among the three PPAR isotypes. Due to its broad tissue distribution, it is difficult, a priori, to foresee a specific function for this receptor. A mouse knockout model, although difficult to generate due to a highly penetrant lethality [41, 44], indicates a role for PPARβ in embryo implantation, as well as in myelination, lipid metabolism and adiposity [44, 45]. In an in vivo skin wound healing model, PPARβ was recently shown to be involved in the control of keratinocyte proliferation, to be necessary for inflammation-induced keratinocyte differentiation and to increase keratinocyte resistance to tumor necrosis factor-alpha (TNF-α)-induced apoptosis [41, 46]. PPARβ can also be activated by peroxisome proliferators and fatty acids to induce gene expression [16, 22], although transcriptional activation by these compounds appears weaker than for PPARα. Interestingly, the activator profile is also biased towards the polyunsaturated fatty acids, which has to do with structural constraints in the LBD and the flexibility provided by the double bonds in the hydrocarbon chain of polyunsaturated fatty acids. In an attempt to identify naturally occurring ligands for PPARβ, acetone extracts of rat tissues were shown to contain an activity that enhances PPARβ-mediated transcription [47]. High-pressure liquid chromatography (HPLC) purification of this activity identified methylpalmitate as a potent activator. This compound, however, also activates the PPARα isotype. Therefore, the hunt for natural selective ligands of PPARβ is still open. There are, however, synthetic molecules able to selectively activate PPARβ. Using a focused combinatorial library, biased towards PPAR binding, it was possible to identify a fibrate derivative, the GW 2433 compound, capable of selectively activating the PPARβ isotype [48]. Two other synthetic compounds, the L165041 (a phenoxyacetic acid derivative) and GW501516 are PPARβ-selective agonists [49, 50]. However, PPARβ shows species-dependent binding characteristics [51]. For instance, bezafibrate is a *Xenopus* PPARβ specific ligand, but its activity is much weaker on the mammalian β isotype than it is on the mammalian α isotype [16].

Knowledge concerning PPARβ functions and identification of PPARβ selective synthetic ligands are still recent. Therefore, the therapeutic potential of this receptor is still unknown. The development of an adequate knockout system for PPARβ and the discovery of the physiological ligands will certainly be of very high interest in the near future.

PPARγ

A more restricted profile of activators distinguishes PPARγ from the two other PPAR isotypes. Indeed, PPARγ is more selective for polyunsaturated fatty acids versus other fatty acids [16, 52]. Another interesting feature is its limited tissue distribution: abundant in adipose tissue, and at much lower levels elsewhere [53, 54]. Using retrovirally transduced cells, it was possible to demonstrate that PPARγ is a major player in the adipocyte differentiation program [55, 56]. Uncommitted fibroblasts can be driven by PPARγ to become adipocytes in a ligand-dependent way (see fig. 2). A ligand-dependent role of PPARγ was also established in the transdifferentiation program of myoblasts to adipocytes [57]. Together, these observations reveal the primordial role of PPARγ in the definition of the adipocytic fate, and its study contributes to a better understanding of the adipocyte lineage within the organism. Furthermore, the finding that synthetic compounds with antidiabetic properties are true PPARγ ligands [58] attracted much attention on this isotype. The thiazolidinediones (TZD) group of molecules (troglitazone, pioglitazone and rosiglitazone/BRL 49653) act positively on insulin action and negatively on glucose levels in the blood. They are able first to bind PPARγ, and then trigger adipogenesis. Moreover, they provide a very plausible link between the regulation of glucose status and PPARγ activity [59]. A possible mechanism by which TZDs reduce insulin resistance is that they direct fatty acids away from skeletal muscle by stimulating their uptake by the adipose tissue. As a result, there would be increased glucose utilization by skeletal muscles. However, it has been observed that mice lacking adipose tissue still benefit from reduction of insulin resistance in response to TZD treatment [60], suggesting that an additional mechanism is at work. Interestingly, L-tyrosine-based insulin sensitizers are also PPARγ ligands, which reinforces the role of PPARγ in insulin resistance [26]. In addition to polyunsaturated fatty acids, the prostanoid 15Δ-prostaglandin J_2 (PG J_2) was identified as a PPARγ ligand [16, 61, 62]. This molecule derives from arachidonic acid, via the cyclooxygenase-2 pathway that produces prostaglandins. It is not clear, however, whether this molecule is the physiological ligand of PPARγ. In terms of natural PPARγ ligands, an interesting model is the one provided by the monocytic lineage [63]. Indeed, PPARγ is well expressed in activated macrophages [64]. In these cells, PPARγ expression is induced by oxidized LDL (oxLDL). Analysis of oxLDL lipid content revealed the presence of two oxygenated derivatives of linoleic

acid, which are 9-HODE and 13-HODE (hydroxyoctadecadienoic acid), two PPARγ ligands. Their affinity for PPARγ is comparable to their precursor, linoleic acid. However, a concerted series of events could favor the action of 9- and 13-HODE as ligands. Indeed, the cell surface scavenger receptor CD36, whose gene is a target of PPARγ [64], is involved in the uptake of oxLDL by the cell, hence providing a source of ligands for PPARγ. Interestingly, the activation of PPARγ seems to be antiatherogenic in macrophages [65–67]. In fact, the internalization of lipids by CD36 is counterbalanced by a PPARγ-mediated upregulation of the reverse cholesterol transporter ABCA1 expression, probably through the enhanced expression of the nuclear receptor LXR. The whole cascade results in a net lipid efflux. It is interesting to note that CD36 is also upregulated during the adipogenic program and that a dynamic lipid flux could occur in these differentiated cells, as it is the case in macrophages.

Finally, an indirect consequence of PPARγ association with the adipocyte differentiation pathway is its potential ability to drive dividing cells towards a differentiated adipocytic state [68]. This can occur in liposarcomas, where PPARγ is expressed. Under the effect of TZD, PPARγ action withdraws these malignant cells from the cell cycle [69]. The overall effect in cell culture is the growth arrest of the sarcoma (see fig. 2). In clinical trials, the administration of TZD to patients suffering from liposarcoma induced a terminal adipocytic differentiation of tumor cells, with the downregulation of cell proliferation markers [70]. Interestingly, the treatment of liposarcoma cells with retinoids enhances the TZD effect, since PPARγ functions as a heterodimer with RXR (retinoid X receptor). A similar situation is observed in breast adenocarcinoma cells, with respect to the ability of PPARγ to reduce cell growth [71]. It will be interesting, in the future, to test whether natural PPARγ ligands are also able to trigger growth arrest in carcinogenic situations. In contrast to these observations, PPARγ agonists were shown to increase the frequency and size of colon tumors in mice bearing a mutation in the adenomatous polyposis colon tumor suppressor gene (APC) [72, 73]. It is possible that depending on the context, PPARγ action could be either beneficial or deleterious. The debate is still open and deserves further study.

Cross-talk with endocrine signaling pathways

The transcriptional activity of PPARs is influenced by the endocrine status of the cell. Clearly, retinoids positively affect PPAR-RXR heterodimer activity by binding to RXR [22, 74]. Inversely, thyroid hormone, which controls some fatty acid metabolism genes via its nuclear receptor (TR), seems to act negatively on PPAR action. An example of this negative effect is the inhibition of ciprofibrate-induced expression of the rat peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase gene by thyroid hormone [75]. Ciprofibrate, which is a fibrate derivative, acts through PPAR to activate transcription. The inhibitory effect of thyroid hormone might involve the titration of RXR, which becomes limiting for PPAR [76]. This functional inhibition could also involve non-RXR interacting molecules which remain to be identified. Another important cellular status for PPAR activity is the fatty acid content of the cell. Indeed, fatty acids and their derivatives participate in transcriptional signaling cascades [77], having more than their traditional structural and energy-providing roles. As mentioned earlier, PPARγ is the nuclear receptor for 15Δ-prostaglandin J2, which derives from PGH2, a cyclooxygenase 2-dependent metabolite of arachidonic acid. PGH2 is also catabolized to PGF2α. This prostanoid is targeted to the outer cell membrane and binds to a G-protein-coupled receptor [78]. Interestingly, PGF2α is able to inhibit PPARγ functions in adipogenesis [79] (see fig. 2). This occurs through PGF2α activation of the MAPK pathway, and subsequent PPARγ phosphorylation and inhibition of its transcriptional activity. A similar cell membrane-nuclear receptor cross-talk occurs with PPARα. Indeed, a membrane receptor for LTB_4 has been cloned [80], and it can share ligands with PPARα [36]. It will be interesting to evaluate whether LTB_4 signaling at the cell membrane is linked to PPARα activity. Finally, the nuclear receptor LXR, which binds cholesterol derivatives, such as the 24(*S*)-hydroxycholesterol [81], is implicated in the regulation of cholesterol homeostasis. LXRα knockout mice show impaired bile acid metabolism under high-cholesterol diet. This defect in LXR eventually leads to a serious alteration in liver function [82]. Since both PPAR and LXR are regulated by diet and interact with RXR to activate transcription, it is not surprising that LXR and PPAR functions are interconnected [83, 84].

Concluding remarks

The exploration of PPAR biology was one of the most fruitful in the world of nuclear hormone receptors. PPARs are implicated in many cellular processes, from cell cycle to cell differentiation, from inflammation to apoptosis, and are crucial for energy homeostasis [25]. The design of selective ligands for each isotype, agonists and antagonists, has been and will be interesting for both fundamental and therapeutic applications. Academic and pharmaceutical research have been complementary in the discovery of new ligands in a sort of ‘pharma cycle’: (i) identification of novel natural ligands by classical means, (ii) design of combinatorial libraries, (iii) in vitro testing, based on induced structural conformational changes, or

other characteristics and (iv) in vivo analyses, using cell-based approaches and animal models. Understanding how eicosanoids can influence PPAR-mediated gene expression will require better understanding of the intracellular and intranuclear concentration and trafficking of these substances. Most fatty acid derivatives are subject to oxidation processes as well as to interaction with transport molecules, which has to be taken into account in receptor-ligand interaction studies. This could also apply for RXR, the heterodimer partner of PPAR. Not least, ligand diversity coupled to subtle differences in the induced modifications of the LBD three-dimensional structure may determine the composition of interacting proteins in the machinery responsible for transcriptional initiation. This diversity would participate in the numerous PPAR functions within the organism.

Acknowledgements. The authors wish to thank Sander Kersten for critical reading of the manuscript, Pascal Escher and Macha Klimek for helping in figure design and Sandra Rojas for fruitful discussions. The work done in the authors' laboratory was supported by the Swiss National Science Foundation, the Etat de Vaud and the Human Frontier Science Program Organization.

1 Auwerx J. (1992) Regulation of gene expression by fatty acids and fibric acid derivatives: an integrative role for peroxisome proliferator activated receptors. The Belgian Endocrine Society Lecture 1992. Horm. Res. **38:** 269–277
2 Desvergne B., Ipenberg A., Devchand P. R. and Wahli W. (1998) The peroxisome proliferator-activated receptors at the crossroad of diet and hormonal signalling. J. Steroid Biochem. Mol. Biol. **65:** 65–74
3 Lemberger T., Desvergne B. and Wahli W. (1996) Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu. Rev. Cell. Dev. Biol. **12:** 335–363
4 Mangelsdorf D. J., Thummel C., Beato M., Herrlich P., Schutz G., Umesono K. et al. (1995) The nuclear receptor superfamily: the second decade. Cell **83:** 835–839
5 Wahli W. and Martinez E. (1991) Superfamily of steroid nuclear receptors: positive and negative regulators of gene expression. FASEB J. **5:** 2243–2249
6 Dreyer C., Krey G., Keller H., Givel F., Helftenbein G. and Wahli W. (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell **68:** 879–887
7 Issemann I. and Green S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature **347:** 645–650
8 Gottlicher M., Widmark E., Li Q. and Gustafsson J. A. (1992) Fatty acids activate a chimera of the clofibric acid-activated receptor and the glucocorticoid receptor. Proc. Natl. Acad. Sci. USA **89:** 4653–4657
9 Sher T., Yi H. F., McBride O. W. and Gonzalez F. J. (1993) cDNA cloning, chromosomal mapping and functional characterization of the human peroxisome proliferator activated receptor. Biochemistry **32:** 5598–5604
10 A unified nomenclature system for the nuclear receptor superfamily. Cell 1999. **97:** 161–163
11 Evans R. M. (1988) The steroid and thyroid hormone receptor superfamily. Science **240:** 889–895
12 Juge-Aubry C. E., Hammar E., Siegrist-Kaiser C., Pernin A., Takeshita A., Chin W. W. et al. (1999) Regulation of the transcriptional activity of the peroxisome proliferator-activated receptor alpha by phosphorylation of a ligand-independent trans-activating domain. J. Biol. Chem. **274:** 10505–10510
13 Hu E., Kim J. B., Sarraf P. and Spiegelman B. M. (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. Science **274:** 2100–2103
14 Schwabe J. W., Neuhaus D. and Rhodes D. (1990) Solution structure of the DNA-binding domain of the oestrogen receptor. Nature **348:** 458–461
15 Kalkhoven E., Valentine J. E., Heery D. M. and Parker M. G. (1998) Isoforms of steroid receptor co-activator 1 differ in their ability to potentiate transcription by the oestrogen receptor. EMBO J. **17:** 232–243
16 Krey G., Braissant O., L'Horset F., Kalkhoven E., Perroud M., Parker M. G. et al. (1997) Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol. Endocrinol. **11:** 779–791
17 Onate S. A., Tsai S. Y., Tsai M. J. and O'Malley B. W. (1995) Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science **270:** 1354–1357
18 Chakravarti D., LaMorte V. J., Nelson M. C., Nakajima T., Schulman I. G., Juguilon H. et al. (1996) Role of CBP/P300 in nuclear receptor signalling. Nature **383:** 99–103
19 Dowell P., Ishmael J. E., Avram D., Peterson V. J., Nevrivy D. J. and Leid M. (1997) p300 functions as a coactivator for the peroxisome proliferator- activated receptor alpha. J. Biol. Chem. **272:** 33435–33443
20 Kamei Y., Xu L., Heinzel T., Torchia J., Kurokawa R., Gloss B. et al. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell **85:** 403–414
21 Gearing K. L., Gottlicher M., Teboul M., Widmark E. and Gustafsson J. A. (1993) Interaction of the peroxisome-proliferator-activated receptor and retinoid X receptor. Proc. Natl. Acad. Sci. USA **90:** 1440–1444
22 Keller H., Dreyer C., Medin J., Mahfoudi A., Ozato K. and Wahli W. (1993) Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. Proc. Natl. Acad. Sci. USA **90:** 2160–2164
23 Kliewer S. A., Umesono K., Noonan D. J., Heyman R. A. and Evans R. M. (1992) Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature **358:** 771–774
24 Heyman R. A., Mangelsdorf D. J., Dyck J. A., Stein R. B., Eichele G. and Evans R. M. (1992) 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell **68:** 397–406
25 Desvergne B. and Wahli W. (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr. Rev. **20:** 649–688
26 Debril M. B., Renaud J. P., Fajas L. and Auwerx J. (2001) The pleiotropic functions of peroxisome proliferator-activated receptor gamma. J. Mol. Med. **79:** 30–47
27 Wurtz J. M., Bourguet W., Renaud J. P., Vivat V., Chambon P., Moras D. et al. (1996) A canonical structure for the ligand-binding domain of nuclear receptors. Nat. Struct. Biol. **3:** 87–94
28 Xu H. E., Lambert M. H., Montana V. G., Plunket K. D., Moore L. B., Collins J. L. et al. (2001) Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proc. Natl. Acad. Sci. USA **98:** 13919–13924
29 Xu H. E., Lambert M. H., Montana V. G., Parks D. J., Blanchard S. G., Brown P. J. et al. (1999) Molecular recognition of fatty acids by peroxisome proliferator- activated receptors. Mol. Cells **3:** 397–403
30 Nolte R. T., Wisely G. B., Westin S., Cobb J. E., Lambert M. H., Kurokawa R. et al. (1998) Ligand binding and co-activator as-

sembly of the peroxisome proliferator-activated receptor-gamma. Nature **395:** 137–143
31 Wagner R. L., Apriletti J. W., McGrath M. E., West B. L., Baxter J. D. and Fletterick R. J. (1995) A structural role for hormone in the thyroid hormone receptor. Nature **378:** 690–697
32 Mascaro C., Acosta E., Ortiz J. A., Marrero P. F., Hegardt F. G. and Haro D. (1998) Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J. Biol. Chem. **273:** 8560–8563
33 Kroetz D. L., Yook P., Costet P., Bianchi P. and Pineau T. (1998) Peroxisome proliferator-activated receptor alpha controls the hepatic CYP4A induction adaptive response to starvation and diabetes. J. Biol. Chem. **273:** 31581–31589
34 Keller H., Mahfoudi A., Dreyer C., Hihi A. K., Medin J., Ozato K. et al. (1993) Peroxisome proliferator-activated receptors and lipid metabolism. Ann. N. Y. Acad. Sci. **684:** 157–173
35 Forman B. M., Chen J. and Evans R. M. (1997) Hypolipidemic drugs, polyunsaturated fatty acids and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc. Natl. Acad. Sci. USA **94:** 4312–4317
36 Devchand P. R., Hihi A. K., Perroud M., Schleuning W. D., Spiegelman B. M. and Wahli W. (1999) Chemical probes that differentially modulate peroxisome proliferator- activated receptor alpha and BLTR, nuclear and cell surface receptors for leukotriene B(4). J. Biol. Chem. **274:** 23341–23348
37 Dowell P., Peterson V. J., Zabriskie T. M. and Leid M. (1997) Ligand-induced peroxisome proliferator-activated receptor alpha conformational change. J. Biol. Chem. **272:** 2013–20120
38 Lin Q., Ruuska S. E., Shaw N. S., Dong D. and Noy N. (1999) Ligand selectivity of the peroxisome proliferator-activated receptor alpha. Biochemistry **38:** 185–190
39 Devchand P. R., Keller H., Peters J. M., Vazquez L. M., Gonzalez F. J. and Wahli W. (1996) The PPARalpha-leukotriene B4 pathway to inflammation control. Nature **384:** 39–43
40 Serhan C. N. (1996) Inflammation. Signalling the fat controller. Nature **384:** 23–24
41 Michalik L., Desvergne B., Tan N. S., Basu-Modak S., Escher P., Rieusset J. et al. (2001) Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. J. Cell Biol. **154:** 799–814
42 Staels B., Koenig W., Habib A., Merval R., Lebret M., Torra I. P. et al. (1998) Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. Nature **393:** 790–793
43 Delerive P., De Bosscher K., Besnard S., Vanden Berghe W., Peters J. M. et al. (1999) Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. J. Biol. Chem. **274:** 32048–32054
44 Barak Y., Liao D., He W., Ong E. S., Nelson M. C., Olefsky J. M. et al. (2002) Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. Proc. Natl. Acad. Sci. USA **99:** 303–308
45 Peters J. M., Lee S. S., Li W., Ward J. M., Gavrilova O., Everett C. et al. (2000) Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta). Mol. Cell. Biol. **20:** 5119–5128
46 Tan N. S., Michalik L., Noy N., Yasmin R., Pacot C., Heim M. et al. (2001) Critical roles of PPARbeta/delta in keratinocyte response to inflammation. Genes Dev. **15:** 3263–3277
47 Schmidt A., Vogel R. L., Witherup K. M., Ruttedge S. J., Pitzenberger S. M., Adam M. et al. (1996) Identification of fatty acid methyl ester as naturally occurring transcriptional regulators of the members of the peroxisome proliferator-activated receptor family. Lipids **31:** 1115–1124
48 Brown P. J., Smith-Oliver T. A., Charifson P. S., Tomkinson N. C., Fivush A. M., Sternbach D. D. et al. (1997) Identification of peroxisome proliferator-activated receptor ligands from a biased chemical library. Chem. Biol. **4:** 909–918
49 Oliver W. R. Jr, Shenk J. L., Snaith M. R., Russell C. S., Plunkett K. D., Bodkin N. L. et al. (2001) A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc. Natl. Acad. Sci. USA **98:** 5306–5311
50 Berger J., Leibowitz M. D., Doebber T. W., Elbrecht A., Zhang B., Zhon G. et al. (1999) Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARdelta ligands produce distinct biological effects. J. Biol. Chem. **274:** 6718–6725
51 Keller H., Devchand P. R., Perroud M. and Wahli W. (1997) PPAR alpha structure-function relationships derived from species- specific differences in responsiveness to hypolipidemic agents. Biol. Chem. **378:** 651–655
52 Kliewer S. A., Sundseth S. S., Jones S. A., Brown P. J., Wisely G. B., Koble C. S. et al. (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc. Natl. Acad. Sci. USA **94:** 4318–4323
53 Braissant O., Foufelle F., Scotto C., Dauca M. and Wahli W. (1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta and -gamma in the adult rat. Endocrinology **137:** 354–366
54 Braissant O. and Wahli W. (1998) Differential expression of peroxisome proliferator-activated receptor- alpha, -beta and -gamma during rat embryonic development. Endocrinology **139:** 2748–2754
55 Tontonoz P., Hu E., Graves R. R., Budavari A. I. and Spiegelman B. M. (1994) mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. **8:** 1224–1234
56 Tontonoz P., Hu E. and Spiegelman B. M. (1994) Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell **79:** 1147–1156
57 Hu E., Tontonoz P. and Spiegelman B. M. (1995) Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. Proc. Natl. Acad. Sci. USA **92:** 9856–9860
58 Lehmann J. M., Moore L. B., Smith-Oliver T. A., Wilkison W. O., Willson T. M. and Kliewer S. A. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J. Biol. Chem. **270:** 12953–1296
59 Willson T. M. and Wahli W. (1997) Peroxisome proliferator-activated receptor agonists. Curr. Opin. Chem. Biol. **1:** 235–241
60 Burant C. F., Sreenan S., Hirano K., Tai T. A., Lohmiller J., Lukens J. et al. (1997) Troglitazone action is independent of adipose tissue. J. Clin. Invest. **100:** 2900–2908
61 Forman B. M., Tontonoz P., Chen J., Brun R. P., Spiegelman B. M. and Evans R. M. (1995) 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell **83:** 803–812
62 Kliewer S. A., Lenhard J. M., Willson T. M., Patel I., Morris D. C. and Lehmann J. M. (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell **83:** 813–819
63 Kliewer S. A. and Willson T. M. (1998) The nuclear receptor PPARgamma – bigger than fat. Curr. Opin. Genet. Dev. **8:** 576–581
64 Nagy L., Tontonoz P., Alvarez J. G., Chen H. and Evans R. M. (1998) Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell **93:** 229–240
65 Chinetti G., Lestavel S., Bocher V., Remaley A. T., Neve B., Torra I. P. et al. (2001) PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat. Med. **7:** 53–58
66 Chawla A., Barak Y., Nagy L., Liao D., Tontonoz P. and Evans R. M. (2001) PPAR-gamma dependent and independent effects

on macrophage-gene expression in lipid metabolism and inflammation. Nat. Med. **7:** 48–52
67 Moore K. J., Rosen E. D., Fitzgerald M. L., Randow F., Andersson L. P., Altshuler D. et al. (2001) The role of PPAR-gamma in macrophage differentiation and cholesterol uptake. Nat. Med. **7:** 41–47
68 Altiok S., Xu M. and Spiegelman B. M. (1997) PPARgamma induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. Genes Dev. **11:** 1987–1998
69 Tontonoz P., Singer S., Forman B. M., Sarraf P., Fletcher J. A. Fletcher C. D. et al. (1997) Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. Proc. Natl. Acad. Sci. USA **94:** 237–241
70 Demetri G. D., Fletcher C. D., Mueller E., Sarraf P., Naujoks R., Campbell N. et al. (1999) Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-gamma ligand troglitazone in patients with liposarcoma. Proc. Natl. Acad. Sci. USA **96:** 3951–3956
71 Mueller E., Sarraf P., Tontonoz P., Evans R. M., Martin K. J., Zhang M. et al. (1998) Terminal differentiation of human breast cancer through PPAR gamma. Mol. Cells **1:** 465–470
72 Saez E., Tontonoz P., Nelson M. C., Alvarez J. G., Minz U. T., Baira S. M. et al. (1998) Activators of the nuclear receptor PPARgamma enhance colon polyp formation. Nat. Med. **4:** 1058–1061
73 Lefebvre A. M., Chen I., Desreumaux P., Najib J., Fruchart J. C., Geboes K. et al. (1998) Activation of the peroxisome proliferator-activated receptor gamma promotes the development of colon tumors in C57BL/6J-APCMin/+ mice. Nat. Med. **4:** 1053–1057
74 Lemberger T., Braissant O., Juge-Aubry C., Keller H., Saladin R., Staels B. et al. (1996) PPAR tissue distribution and interactions with other hormone-signaling pathways. Ann. N. Y. Acad. Sci. **804:** 231–251
75 Chu R., Madison L. D., Lin Y., Kopp P., Rao M. S., Jameson J. L. et al. (1995) Thyroid hormone (T3) inhibits ciprofibrate-induced transcription of genes encoding beta-oxidation enzymes: cross talk between peroxisome proliferator and T3 signaling pathways. Proc. Natl. Acad. Sci. USA **92:** 11593–11597
76 Juge-Aubry C. E., Gorla-Bajszczak A., Pernin A., Lemberger T., Wahli W., Burger A. G. et al. (1995) Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. J. Biol. Chem. **270:** 18117–18122
77 Clarke S. D., Baillie R., Jump D. B. and Nakamura M. T. (1997) Fatty acid regulation of gene expression. Its role in fuel partitioning and insulin resistance. Ann. N. Y. Acad. Sci. **827:** 178–187
78 Sugimoto Y., Hasumoto K., Namba T., Irie A., Katsuyama M., Negiski M. et al. (1994) Cloning and expression of a cDNA for mouse prostaglandin F receptor. J. Biol. Chem. **269:** 1356–1360
79 Reginato M. J., Krakow S. L., Bailey S. T. and Lazar M.A. (1998) Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor gamma. J. Biol. Chem. **273:** 1855–1858
80 Yokomizo T., Izumi T., Chang K., Takuwa Y. and Shimuzu T. (1997) A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. Nature **387:** 620–624
81 Lehmann J. M., Kliewar S. A., Moore L. B., Smith-Oliver T. A., Oliver B. B., Su J. L. et al. (1997) Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J. Biol. Chem. **272:** 3137–3140
82 Peet D. J., Turley S. D., Ma W., Janowski B. A., Lobaccaro J. M., Hammer R. E. et al. (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell **93:** 693–704
83 Peet D. J., Janowski B. A. and Mangelsdorf D. J. (1998) The LXRs: a new class of oxysterol receptors. Curr. Opin. Genet. Dev. **8:** 571–575
84 Chawla A., Boisvert W. A., Lee C. H., Laffitte B. A., Barak Y., Joseph S. B. et al. (2001) A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol. Cells **7:** 161–171