Peroxisome-proliferator-activated receptors as physiological sensors of fatty acid metabolism: molecular regulation in peroxisomes

N. Latruffe1, M. Cherkaoui Malki, V. Nicolas-Frances, B. Jannin, M.-C. Clernencet, F. Hansmannel, P. Passilly-Degrace and J.-P. Berlot

Laboratoire de Biologie Moléculaire et Cellulaire (LBMC), University of Burgundy, 6 boulevard Gabriel, 21000 Dijon, France

Abstract
The enzymes required for the β-oxidation of fatty acyl-CoA are present in peroxisomes and mitochondria. Administration of hypolipidaemic compounds such as clofibrate to rodents leads to an increase in the volume and density of peroxisomes in liver cells. These proliferators also induce simultaneously the expression of genes encoding acyl-CoA oxidase, enoyl-CoA hydratase-hydroxyacyl-CoA dehydrogenase (multifunctional enzyme) and thiolase (3-ketoacyl-CoA thiolase). All these enzymes are responsible for long-chain and very-long-chain fatty acid β-oxidation in peroxisomes. Similar results were observed when rat hepatocytes, or liver-derived cell lines, were cultured with a peroxisome proliferator. The increased expression of these genes is due to the stimulation of their transcription rate. These results show that the peroxisome proliferators act on the hepatic cells and regulate the transcription through various cellular components and pathways, including peroxisome-proliferator-activated receptor α (PPARα). After activation by specific ligands, either fibrates or fatty acid derivatives, PPARα binds to a DNA response element: peroxisome-proliferator-responsive element (PPRE), which is a direct repeat of the following consensus sequence: TGACCTXTGACCT, found in the promoter region of the target genes. PPARα is expressed mainly in liver, intestine and kidney. PPARα is a transcriptional factor, which requires other nuclear proteins for function including retinoic acid X receptor (RXRα) and other regulatory proteins. From our results and others we suggest the role of PPARα in the regulation of the peroxisomal fatty acid β-oxidation. In this regard, we showed that although PPARα binds to thiolase B gene promoter at -681 to -669, a better response is observed with hepatic nuclear factor 4 (‘HNF-4’). Moreover, rat liver PPARα regulatory activity is dependent on its phosphorylated state. In contrast, a protein-kinase-C-mediated signal transduction pathway seems to be modified by peroxisome proliferators, leading to an increase in the phosphorylation level of specific proteins, some of which have been shown to be involved in the phosphoinositide metabolism.

Introduction
Fatty acid β-oxidation, together with that of glucose, is an important pathway to provide cellular energy (in the ATP form) by producing reducing equivalents (NADH) and also to release large amount of acetyl-CoA, a precursor for several important pathways including cholesterol synthesis, farnesyl pyrophosphate and geranylgeranyl pyrophosphate precursors needed for Ras protein–membrane anchoring, and dolichol for glycosylation. Moreover, very-long-chain fatty acid β-oxidation in peroxisomes is considered to regulate the level of arachidonic acid indirectly as a precursor of eicosanoids. Peroxisomes are ubiquitous organelles originally discovered by Rhodin in 1954 [1] and first isolated and characterized by De Duve’s group in 1965 [2]. They appear as membrane-bound vesicles (Figure 1) and contain numerous catabolic and anabolic processes.

Key words: peroxisome, fatty acids, β-oxidation.

Abbreviations used: PI-3K, phosphoinositide 3-kinase; PKC, protein kinase C; PPAR, peroxisome-proliferator-activated receptor; PPRE, peroxisome-proliferator-responsive element; RXR, retinoic acid X receptor.

1To whom correspondence should be addressed (e-mail latruffe@u-bourgogne.fr).
enzymes. Peroxisome function impairment leads to severe diseases such as Refsum and Zellweger syndromes [3]. Whereas studies on yeast show an almost exclusive dependence on peroxisome for fatty acid β-oxidation, in mammalian species this pathway occurs both in mitochondria and in peroxisomes. The contribution of peroxisomes to the oxidation of long-chain and very-long-chain fatty acids relative to that of mitochondria can increase up to 50% in rat liver for C16 or longer-chain fatty acids [4]. Interestingly, peroxisomes and mitochondria are often located in the immediate vicinity of lipid droplets, confirming the close relationship between the intracellular location and the complementing functions of these two organelles (Figure 1).

Rat and mouse liver peroxisomes are able to undergo a high degree of proliferation [5]. This unique property is triggered by a large variety of chemicals of the peroxisome proliferator family, including hypolipidaemic agents (fibrates). This phenomenon is species specific and virtually restricted to rodents because primates (including humans) are non-responders [6].

During peroxisome proliferation, fibrates stimulate the transcription of genes encoding enzymes of lipid metabolism, especially of fatty acid β-oxidation [7] in a peroxisome-proliferator-activated receptor α (PPARα)-dependent manner. Moreover, PPARα function is modulated by its phosphorylation state and the response to peroxisome proliferators is dependent on a signalling cascade from the membrane to the nucleus, where several kinases have been identified. This might lead to concerted regulation between fatty acid and glucose metabolism.

**Properties of PPAR**

PPARα was discovered 10 years ago by Issemann and Green [8]; subsequently, Dreyer et al. [9] cloned two other members of this subfamily [β(δ)] and γ[ belonging to the steroid receptor superfamily. PPARs are now considered to be essential transcription factors regulating key cellular functions including lipid metabolism, xenobiotic metabolism, inflammation, cell differentiation and cancer (Figure 2). Their tissue distribution is well documented [10]: PPARα is largely expressed in liver and intestine and slightly or not at all in other tissues (Figure 2). In contrast, PPARγ is expressed mainly in adipose tissue and less in intestine, whereas PPARβ(δ) seems to be a ubiquitous
transcription factor. Subsequently, PPARs were shown to be activated by the binding of specific ligands including peroxisome proliferators [11]. In particular, PPARs are activated by either pharmacological or physiological ligands [12]. The pharmacological ligands are the fibrates (hypolipidaemic drugs) for PPARα and the thiazolidinediones (antidiabetic drugs of type II diabetes) for PPARγ. The biological ligands belong mostly to signalling messenger molecules produced from the arachidonic cascade pathways, namely the leukotriene LTB4 of the lipoxygenase route for PPARα or the prostaglandin Δ15PGJ2 of the cyclo-oxygenase route for PPARγ. In contrast, PPARβ/δ is preferentially activated by unsaturated fatty acids [13]. To elucidate the interactions between ligands and PPARs or other peroxisome binding proteins, we developed a new probe, a fluorescent bezafibrate derivative, DNS-X [14]. This molecule is able to trigger peroxisome proliferation both in vitro [14] and in vivo [15] and to undergo a PPARα activation by transfection assays. The PPAR-DNA response element is a direct repeat motif with a consensus sequence of TGACCT(T/A)TGACCT [16]. Such a peroxisome-proliferator-responsive element (PPRE) is found in the promoter of all the genes that encode peroxisomal fatty acid β-oxidation enzymes.

With others, we showed that the PPAR–PPRE interaction leads to transcriptional activation [17]. For this to occur, PPARα must be heterodimerized with retinoic acid X receptor (RXRα), another transcription factor activated by 9-cis retinoic acid.

**PPARα-dependent transcriptional activation of genes encoding peroxisomal fatty acid β-oxidation enzymes**

In rat liver, the level of these specific mRNAs is strongly increased after treatment with the peroxisome proliferator ciprofibrate [7]. Indeed this high level of mRNAs is observed for the whole metabolic pathway, including acyl-CoA synthetase, the membrane ABC transporter, ALDRP (adrenoleukodystrophy related protein), acyl-CoA oxidase, the multifunctional enzyme (hydratase–dehydrogenase), thiolase (3-ketoacyl-CoA thiolase) and acetyl/octanoyl carnitine transferases (Figure 2). To some extent this overall transcription increase is reminiscent of bacterial operon induction. Recently we have been interested in the regulation of the thiolase B gene. This gene is strongly activated by peroxisome proliferators [18] and shows a PPRE in its promoter (Figure 3) at position −669 to −681 [19]. Thiolase is the last enzyme of the fatty acid β-oxidation process producing acetyl-CoA and a fatty acid shortened by two carbons per cycle. Although PPARα interacts with thiolase B PPRE, the binding and the transcriptional effects of HNF-4 are stronger.
on the promoter [20], indicating a discrepancy between thiolase and the other two enzymes encoded by genes of the β-oxidation pathway.

**Modulation of PPARα activity by phosphorylation**

Among the various possibilities of protein modulation i.e. transcriptional and translational regulation, ligand-mediated allosteric changes and protein–protein interactions, the phosphorylation/dephosphorylation process is an important mechanism of modulation, which is also valid for transcription factors [21]. Indeed, PPARα is phosphorylated in rat Fao hepatoma cells [22]. This phosphorylation is enhanced by cell treatment with ciprofibrate, a peroxisome proliferator. The consequence of PPARα phosphorylation on a peroxisome marker, the acyl-CoA oxidase encoded by a PPARα target gene, is a decrease in acyl-CoA oxidase activity [22]. Other reports showed that a MAP-kinase-dependent phosphorylation occurs at least at the serine-12 located at the N-terminal region A/B transactivating domain [23]. Moreover, the phosphorylation of PPARα is insulin dependent [24] and PPARα activity is modulated by an inhibitor under the control of a kinase complex regulated by growth hormone [25]. Interestingly, there is similar phosphorylation of PPARγ in preadipocytes/adipocytes, where PPARγ phosphorylation is dependent on MAP kinase and leads to a decrease in PPARγ-mediated preadipocyte differentiation into adipocytes [26].

**Protein kinase C (PKC) mediates peroxisome proliferator effects**

Until recently there was little information on the effect of peroxisome proliferators on signal transduction. Several years ago it was reported that PKC was stimulated in vitro by peroxisome proliferators [27]. We reported an increase in phosphorylation of several cellular phosphoproteins in the presence of ciprofibrate [28]. Recently, by using Fao-permeabilized cells, we reported that ciprofibrate in vitro stimulates the PKC-dependent phosphorylation of a specific substrate [29]. In contrast, in cells pretreated with ciprofibrate, a particulate fraction contains both kinase activity and kinase substrate because the 32P labelling in vitro leads to an increase in the phosphorylation band 85 kDa of protein, in agreement with the implication of PKC [29].

Attempts to identify the p85 polypeptide strongly suggest an identity with a regulatory component of the phosphoinositide 3-kinase (PI-3K) having a molecular mass of 85 kDa [30] (J. Riusset, H. Vidal, P. Pasilly, B. Jannin and N. Latruffe, unpublished work). In this case the ciprofibrate-dependent activation of PKC would recruit both p85 (the regulatory subunit) and p110 (the catalytic subunit) of PI-3K [31] from the membrane to allow the activation of protein kinase B (PKB = Akt) leading to the triggering of the phosphoinositide signalling cascade [32]. This mechanism would be consistent with a concerted and reciprocal regulation of glucose and fatty acid metabolism [33]. Whereas fatty acid β-oxidation

---

**Figure 3**

**Structure of rat peroxisome thiolase B gene promoter**

The Figure was adapted with permission from [19] (1990) © The American Society for Biochemistry & Molecular Biology.
reactions would be stimulated through the activation of PPARz by fibrate, diversion of glucose metabolism into glycogenogenesis occurs through activation of the PI-3K cascade, involving PKC and p85 [34].

Finally, other evidence suggests that the level of ciprofibrate-sensitive thiolase mRNA is markedly decreased when cells are exposed to PKC inhibitors [35]. This finding strongly supports the view that fibrate-modulated PKC activity might be important in the regulation of peroxisomal fatty acid β-oxidation.

Conclusions

The peroxisomal fatty acid β-oxidation pathway is regulated by peroxisome proliferators, especially by hypolipidaemic agents of the fibrate family through PPARz, which can be modulated by a ligand-activated process, by covalent phosphorylation and through signal transduction mechanisms involving mitogen-activated protein (‘MAP’) kinase, PKC and, possibly, PI-3K.

We thank Dr Jean-Pierre Zahnd (Faculty of Medicine, Dijon, France) and Dr Paul Deslex (Laboratoires Sterling-Winthrop, Dijon, France) and their teams for collaboration [35] and for performing electron microscopy of guinea-pig liver; and Dr Salish Surapureddi for critical reading of the manuscript. This programme is supported by the Regional Council of Burgundy and the Cancer League of Burgundy.

References