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Review

Genetic-dependency of peroxisomal cell functions – emerging aspects

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Abstract

This paper reviews aspects concerning the genetic regulation of the expression of the well studied peroxisomal genes including those of fatty acid β-oxidation enzymes; acyl-CoA oxidase, multifunctional enzyme and thiolase from different tissues and species. An important statement is PPARα, which is now long known to be in rodents the key nuclear receptor orchestrating liver peroxisome proliferation and enhanced peroxisomal β-oxidation, does not appear to control so strongly in man the expression of genes involved in peroxisomal fatty acid β-oxidation related enzymes. In this respect, the present review strengthens among others the emerging concept that, in the humans, the main genes whose expression is up-regulated by PPARα are mitochondrial and less peroxisomal genes. A special emphasis is also made on the animal cold adaptation and on need for sustained study of peroxisomal enzymes and genes; challenging that some essential roles of peroxisomes in cell function and regulation still remain to be discovered.

Keywords: peroxisome - gene - β-oxidation - biogenesis - PPAR - fibrates - fatty acids - hormones

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Introduction

Microbodies, which include peroxisomes, contribute to key metabolic pathways of the cell function. In some species, especially in rodents, these organelles are able to proliferate abundantly upon exposure to so-called peroxisome proliferators. Peroxisome-located enzymes are also regulated by many molecules including nutrients (fatty acids, steroids), hormones (T3, retinoids), PPARs and other nuclear signaling factors. The present review covers the following aspects: 1 – Historical background, 2 – Recall on lipid metabolism and peroxisome metabolic pathways, and currently unresolved issues; 3 – Regulation of peroxisomal enzymes and strategic approaches for their study (Biological models, tissue – organs, investigating domains, methodologies and physiological situations); 4 – Disorders with peroxisomal gene altered functions; 5 – Sustained study of key peroxisomal enzymes and genes; 6 – Conclusions and perspective. For valuable consideration on peroxisomal disorders and regulation of genes the reader may kindly refer to the accounts of a recent symposium [1].

Historical background

The recognition of inborn errors affecting peroxisome enzymes and biogenesis has actually boosted our knowledge on the function of peroxisomes in mammalian cells. Biological and biochemical findings that may be encountered in human peroxisomal disorders include deficient plasmalogen biosynthesis, reduced common C24 bile acid formation with excess C27 bile acid precursors, reduced cholesterol, and very long-chain fatty acid (VLCFA) accumulation in body fluids and tissues. These findings have highlighted the role of peroxisomes which appear to be essential for numerous metabolic functions (see Table 1 and next section).

Presently, as far as we know, the amount of peroxisomal enzymes are mostly controlled at the gene transcriptional level. This gene regulation is especially very large in liver from rodent species and may be dramatically amplified by the so-called peroxisome proliferation phenomena. In fact, our understanding of this regulation results largely from studies on PPARs (Peroxisome Proliferator-Activated Receptors) and other nuclear receptors including HNF4 (Hepatic Nuclear Factor n°4). Other signaling transcription factors have been shown to modulate peroxisomal gene expression, i.e. thyroid hormone receptors, glucocorticoids receptors, LXR. It has been also well documented that additional cofactors (often tissue specific) are also required for regulation of the nuclear transcriptional machinery. On the other hand, specific ligands whose prototypes such as fibrates were historically long known for their hepatomegaly and peroxisome proliferation inductive properties in the rodents before PPARα was discovered [2], are required for triggering nuclear receptor activation. These ligands (or regulatory molecules) can be either dietary compounds or metabolites (nutrients like fatty acids, cholesterol or cholesterol derivatives), biological signaling molecules (thyroid hormones, glucocorticoids, retinoids) or pharmacological compounds (fibrates, dexamethasone).

Table 1. Peroxisome metabolic pathways.

- Oxidation (with H_2O_2 production) of
  1. VLCFA, LCFA, PUFA
  2. Prostaglandins
  3. Biliary salts
  4. Polyamines
  5. Amino acids
  6. α-hydroxy acids
  7. Purines
- Plasmalogen synthesis
- Cholesterol synthesis

Lipid metabolism and peroxisome metabolic pathways. Current challenges

Cell and body fatty acid metabolism involves three main categories of molecules, i.e. triglycerides, fatty acids and lipoproteins, and several pathways including trafficking from white adipose tissue to liver which exhibits intracellular sites for fatty acid breakdown : mitochondria, peroxisomes and endoplasmic reticulum. The protein content and function of peroxisomes have been provided with a better under-

standing and characterization consequently to the recognition of inborn errors affecting peroxisome enzymes and biogenesis. The role of peroxisome includes catalysis of numerous metabolic functions (Table 1) among which fatty acid transport and β-oxidation (VLCFA, PUFA-Poly Unsaturated Fatty Acids and derivatives such as arachidonic acid, prostaglandins and leukotrienes), fatty acid β-oxidation (branched-chain fatty acids such as phytanic acid which gives rise to pristanic acid), purine, D-aminoacid and α-hydroxyacid oxidations, polyamine (spermine, spermidine) breakdown [3]. The role of these organelles further extents to anabolic pathways such as initiation of ether lipid/plasmalogen biosynthesis, cholesterol and bile acid synthesis [3]. Recently, peroxisomes have been shown to be involved in docosahexaenoic acid (DHA) formation [4], and rat liver iNO synthase has been detected in these organelles [5]. Interestingly enough, a decrease in cultured fibroblasts of peroxisome abundance, unrelated to deficiency of a key step in peroxisome biogenesis, is linked to AOX deficiency or defective MFP1 import [6], suggesting that local metabolism might be involved in organelle formation.

At the beginning of this new century, valuable issues which surely deserve to be addressed include: restoration of impaired peroxisomal functions, search for the role of peroxisomes in brain, biological significance of peroxisomes diversity, understanding peroxisomes in interaction with other organelles, involvement of peroxisomes in developmental stages, discovery of currently unexplored peroxisomal functions, and evolutionary aspects of peroxisomes. [7]. In this respect, recently emerging exciting findings related to peroxisome and development have been obtained in lower eukaryotic species [8] where Petriv *et al*. reported that in *C.elegans* gene inactivations of membrane ABC transporters, peroxins involved in the peroxisome biogenesis process (PEX5, PEX12 and PEX19) and metabolic enzymes (dienoyl-CoA isomerase and dihydroxyacetonephosphate synthase) shut up the worms in the early development stage (L1, L2). Using *Podospora anserina*, Berteaux-Lecellier *et al*. [9] have shown that the pex2 mutant is unable of transition between mitotic state and differentiated state, an obligate critical step in the reproductive cycle. Moreover, transfection of PEX2 mutant with human cDNA encoding PMP70 (a peroxisomal membrane protein) restores not only the assembly of peroxisomes but also the normal course of the differentiation commitment process [10].

Exploratory procedures and regulations of peroxisomal enzymes; animal models, inducing factors, activated receptors

Experimental models currently available for investigative explorations of peroxisomes

The animal species and the cell type

A large variety of species have been tested as biological models to reveal either common properties or, on the opposite, specific differences in peroxisome biochemistry and regulation. Rodents (rat, mouse, guinea pig, hamster, etc.) have been largely investigated along with, when possible, human, monkey and other animals. For instance, the comparison of species liver sensitivity has allowed to conclude that human liver cells are resistant to peroxisome proliferators [11]. The gerbil (*Jaculus orientalis*), a rodent from the sub desert highland of Morrocco has successfully avered to exhibit unique peroxisome regulatory properties in response to fibrate [12], cold adaptation and hibernation [13]. Such models are helpful to investigate the extent of adaptive changes in peroxisomal enzymes upon extreme physiological conditions. On the other hand, single cells (cell lines, primary cultures, yeast/fungi), invertebrates (*C.elegans*), and also plant (*A. thaliana*) have been often used for the study of peroxisome.

Relationships between peroxisomes and cell differentiation state have been documented in various tissues and organs notably in liver, vs brain, kidney, muscle, white and brown adipose tissues. Among others, the acyl-CoA oxidase specific activity of human glioblastoma L1 cells increases upon exposure to perfluorododecanoic acid, a peroxisome proliferator, in a cell cycle number dependency [14].

The molecular biology tools and analytical procedures

Standard experimental procedures include enzyme/protein immunoblotting, gene organisation, transcriptional and post-trancriptional investigations

Table 2. Genetic models.

Mouse invalided genes related to peroxisome fatty acid metabolism			
ALDP	Kobayashi's lab	1997	[15]
AOX	Reddy's lab	1996	[16]
MFP1	Reddy's lab	1999	[17]
MFP2	Baes's lab	2000	[18]
TH-SCP2	Seedorf's lab	1998	[19]
TH (inducible)	Latruffe's lab	unpublished yet	
PPAR	Gonzalez's lab	1995	[20]
Other KO genes			
PEX5	Baes's lab	1997	[23]
PEX2	Faust's lab	1997	[24]
PEX5 conditional	Baes's lab	2002	[25]
PEX13 conditional	Bjorkman's lab	2002	[26]
PPAR	Wahli's lab	2001	[21]
PPAR	Gonzalez's lab	2002	[22]
Transgenic mice overexpressing peroxisomal enzyme			
Catalase	Glauert's lab	1996	[27]

(nuclear receptors), biochemical signaling explorations, micro-morphology. Recently, emerging global procedures (genomics, transcriptomics and proteomics screenings) and *in vivo* inactivation of targeted genes (null mice and transgenic models) have been also adapted to study peroxisomes In this respect, a growing up number of mice with KO genes are currently available in and to research laboratories for most of the β-oxidation enzymes, the three PPARs isoforms, several peroxisomal membrane proteins and peroxins (Table 2). Presently, KO mice provide bi-allelic selective inactivation of the following genes (the given list being only indicative and far from being exhaustive): ALDP-/- [15], AOX-/- [16], MFP1-/- [17], MFP2-/- [18], TH-SCP2-/- [19], PPARα -/- [20], PPARβ-/- [21], PPARγ-/- [22], and the peroxins PEX5-/- [23], PEX2-/- [24], PEX5-/- [25], PEX13-/- [26]. There are also available overexpressed gene models which are, however, to our knowledge, currently limited to catalase transgenic mice [27].

Applications in peroxisomal regulation of RNA interference strategy (see [8]) and structural biochemistry are just emerging issues. It has been recently shown that interactions of C-ter and N-ter domains of peroxisomal MFP1 were required for the hydratase-isomerase activity of this protein [28]. Moreover, a throughput cDNA microarray screening of PPAR-target genes in mouse liver has revealed that among 7500 genes/expression tags, 246 were at least two-fold overexpressed in mice given Wy 14.643, a peroxisome proliferator, including PEX11 gene, encoding a peroxisomal membrane protein involved in fatty acid oxidation and peroxisome biogenesis, whose expression was enhanced almost 7-fold [29].

Peroxisome proliferation and nuclear receptor activation

Inductive properties of nutrients, hormones and drugs

As mentioned above, activation of PPARs is triggered by specific ligands which correspond to nutrients/metabolites, hormones or drugs. The use of controlled physiological conditions and precise experimental protocols have been helpful in characterizing regulatory properties of peroxisomes regarding fat, sugar, diabete-obesity, starvation and special diets. After having documented effects in liver, Zipper [30] reported moderate peroxisome proliferation in rat myocardium upon ethanol, physical exercise and C22:1 erucic acid. Fan *et al.* [31] described increased PPARα levels associated with increase fatty acid content in liver from mice with disrupted expression of peroxisomal acyl-CoA oxidase gene. The focus on receptors acting as sensors of the nutritional-endocrinological-pharmacological environment/status emphasizes key roles of thyroid and steroid hormone receptors, PPARs and their ligands (fibrates and other classes of compounds inducing peroxisome proliferators in rodents), retinoids as RXR ligands. Nutritional regulation of metabolic genes is illustrated by stimulatory effects of fatty acids on LXRα gene expression [32], activation of PPARα upon hydroxylated soybean oil [33], by conjugated linoleic acid [34] or by phytanic acid [35]. General inducible and inductive properties of PPARs have been the topic of many reviews, including [36]. A special attention has been paid to PPARα as a possible physiological sensor in man. Suppression of HNF-4α activity by CoA esters of hypolipidaemic peroxi-

some proliferators may account for hypolipidaemic properties of these compounds in the humans independently of PPARα activation by their respective unesterified carboxylate forms. As a consequence, hypolipidaemic activity of so-called peroxisome proliferators is currently suggested to be mediated by PPARα and HNF-4α pathways in rats and man, respectively.

Inductive properties of physiological conditions (cold exposure and hibernation)

Jerboa, one of the deep hibernators originated from sub-desert highlands, represents an excellent model to study during cold adaptation process lipid metabolism. Morphological analysis of liver peroxisomes by electron microscopy reveals a strong alteration in shape and size of this compartment. Cold exposure of *jerboa* results in peroxisome proliferation with an increase in their number and size (+61%). During hibernation only the size becomes large, forming giant peroxisomes with a decrease of their number in hepatocytes [37].

We reported previously [13] that the protein yield of the purified peroxisomal fraction per gram of liver decreased by 60% in both pre-hibernating and hibernating *jerboa*. Taking into account the observed increase in size of peroxisomes in these two physiological states, the decrease in the protein yield might be explained by a shift in peroxisome density that implies the modification of the sedimenting properties. A similar explanation was proposed regarding cold adaptation in rat [38].

It has been reported that white adipose tissue may participate in selective retention of essential fatty acids during the pre-hibernating period [39]. Nedergaard *et al.* [40], reported a 10-fold increase of brown adipose peroxisomal β-oxidation in cold adapted rat and calculated up to 10 % of total brown fat proteins to be peroxisomal. However, recent experiments on rat after 4 weeks cold exposure Guardiola-Diaz *et al.*, [41] show neither significant increase in brown adipose peroxisome proliferation, nor induction of peroxisomal enzymes during the first two weeks of cold adaptation, a period corresponding to the initial proliferative phase taking place in brown adipose tissue. In the same experiment a 7-fold increase in AOX content and peroxisome volume was observed in rat liver [41].

Nuclear signaling factors

The state of the art regarding fatty acid oxidation and biological significance of PPARs in man is not clear yet due to the fact that although human PPARα is functional [42] and positively control peroxisomal fatty acid oxidation enzymes in human HepG2 cell lines [43], there is a low control by overexpression of mouse PPARα in human cells. In contrast, in these circumstances, mitochondrial counterpart is subject to up-regulation [43, 44]. In this line, PPARα activates human muscle carnitine palmitoyltransferase I (CPT I) [45]. On the other hand, PPARs agonists has been shown to repress human cytochrome CYP4F2-LTB4 α-hydroxylase promoter [46].

Interactions between PPARα and other nuclear receptors

There are multiple interactions between PPARα and other nuclear receptors in man and rodents. The thyroid hormone receptor inhibits activation of PPARs by ciprofibrate [47]. LXRα (Liver-X-Receptor) antagonizes mouse PPARα/RXRα-mediated transcriptional activation by peroxisome proliferators [48]. Bile acids inhibit the effects of PPARα activation in part *via* impaired recruitment of transcriptional co-activators [49]. The complex CARα/RXRα (Constitutive Androsterone Receptor/Retinoid X Receptor) recognizes the DR2 element Hydratase-Dehydrogenase PPRE [50]. The nutritional regulation of metabolic genes is illustrated with the modulating effect of fatty acids; i.e. the LXRα gene expression is stimulated by fatty acids [32], the hydroxylated soybean oil stimulates PPARα expression [33] or the conjugated linoleic acid activates PPARα [34].

Cross-talks between PPARs and other nuclear factors

PPARα is also involved in many cross-talks with other nuclear receptors, for instance: PPAR β (δ) inhibits PPARα and its effects on PPRE through the binding of SMRT (Silencing Mediator for Retinoid and Thyroid hormone receptors) and SHARP (SMRT and Histone deacetylase Associated Repressor Protein) [51]. A short heterodimer PPAR-RXR lacking DNA binding activates PPARα-RXRα dependent AOX and MFP1 genes [52]. STAT5b negatively regulates PPARα

Table 3. Peroxisome inherited genetic diseases.

Assembly deficiences	Single peroxisomal enzyme deficiencies
Zellweger syndrome* (PEX1, PEX2, PEX5, PEX6 …)	X-adrenoleukodystrophy*(ALDP) / adrenomyeliloneuropathy*
Neonatal adreno-leukodystrophy* (PEX1, PEX5, PEX6 …)	Acyl-CoA oxidase deficiency* Bifunctional enzyme deficiency*
Infantile Refsum disease* (PEX1, PEX12 …)	DHAP acyltransferase deficiency* Alkyl-DHAP synthase deficiency*
Hyperpipecolic acidemia*	Glutaric acidura III*
Rhizomelic chondrodysplasia punctata* (PEX7 …)	Classical Refsum disease* (Phytanoyl-CoA hydrolase) Hyperoxaluria type I (Alanine-glyoxylate aminotransferase) Acatalasemia

* Nervous System Affected

Adapted from Powers & Moser [63]

[53]. RIP140 negatively interacts with PPARα and LXRα [54]. P300 activates PPARα and PPAR β (δ) in Caco2 cells [55], or PRIC285 (Peroxisome proliferator-activated-Receptor alpha-Interacting factor Complex) functions as a coactivator for PPARα [56].

Cross-talks between PPARs and general cell signaling pathways

Cross-talks of PPAR isoforms with the general cell signaling tranduction pathways have been further documented [57]. The protein kinase A activates PPARα through a higher stability of the PPAR phosphorylated form-DNA complex in mouse [58]. The P38-MAP kinase activates PPARγ through an increase of PGC-1 coactivator binding to PPARγ in mouse myocytes [59]. TNFα inhibits PPARα gene expression and PPARα peroxisomal target genes in rat liver [60].

Interactions of PPARs with gene products other than transcriptional or cell signaling factors

Some target gene products self activate PPAR. For instance, HMG-CoA synthase regulates gene expression by association with PPARα [61] or by interaction of the N-terminus of PPARα with *in vitro* translated bifunctional enzyme [62]. Recently, cytosolic FABP (Fatty Acid Binding Protein) has been shown to interact with PPARs and to relocate the receptor in the nucleus [63].

Disorders with peroxisomal gene altered functions

Several peroxisome inherited genetic diseases affect either organelle biogenesis, components assembly and/or metabolism based peroxisomal enzymes (Table 3). The main disorders characterized by failure to assemble correctly peroxisomes are the cerebro-hepato-renal syndrome or Zellweger's disease, the neonatal adreno-leukodystrophy, the infantile Refsum disease, the hyperpipecolic acidemia, and rhizomelic chondrodysplasia punctata. Single peroxisomal enzyme deficiencies include X-adrenoleukodystrophy (ALDP) / adrenomyeloneuropathy, acyl-CoA oxidase deficiency, bifunctional enzyme deficiency, the DHAP acyltransferase deficiency, alkyl-DHAP synthase deficiency, glutaric acidura type III, classical refsum disease (phytanoyl-CoA hydrolase),

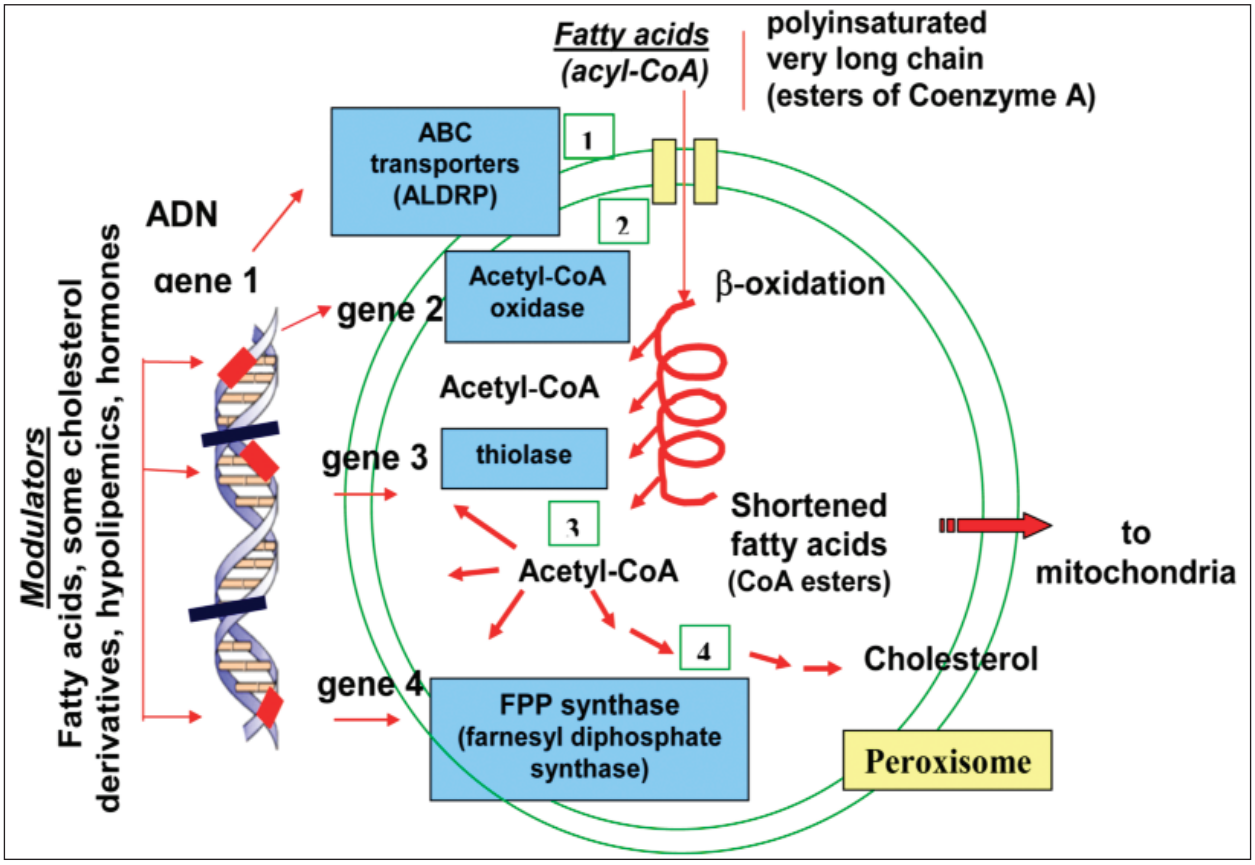

Fig. 1 Key peroxisomal enzymes.

primary hyperoxaluria type I (alanine-glyoxylate aminotransferase) and acatalasemia [64]. In contrast, there are several peroxisomal enzymes for which selectives deficiencies has not been described yet.

This class of diseases impairing peroxisome assembly and/or metabolism may seriously affect patients; they however represent unique experiments of the nature and are helpful in understanding and unravelling many aspects of peroxisome function (for a recent valuable review emphasizing metabolic aspects such as for instance fatty oxidation in these disorders, see [65]). By contributing to elucidate the regulation and the role of peroxisomal genes in human, the study of the molecular basis of these deficiencies may have direct and indirect clinical implications for human health and disease, identifying new targets and mechanisms for pharmacological developments.

Sustained study of key peroxisomal enzymes and genes

Fig. 1 illustrates an example of research on the dialogue between the organelle and cellular genome and its regulation, corresponding to the strategy adopted at the LBMC from the Burgundy University. This research actually focuses on four peroxisomal proteins: the membrane ABC transporters [66], the bifunctional enzyme [67] and PPAR dependent enzymes [68], the farnesyl pyrophosphate synthase [69] and the inducible thiolase (thiolase B). Cholesterol regulates the FPP synthase, an important step on the peroxisome cholesterol synthesis pathway, through SREBP (Sterol response Element Binding Protein) as transcription factor. A key role played by peroxisomal thiolases in cell metabolism, contributing to diverse biochemical pathways including cellular biosyntheses of dolichol, cholesterol, fatty acid

and isoprenoids, protein acylation and energy release. Among others, the research strategy depicted in Figure 1 has recently allowed to show that the promoter-located PPRE of the peroxisomal thiolase B interacts not only with PPARα but also with HNF-4 which activated luciferase gene expression driven by the putative thiolase PPRE [70]. As a suggestion, thiolase B gene induction by peroxisome proliferators might be mediated *via* either recruitment of other gene regulatory elements or *via* additional PPRE sequence(s), all of which remain to be determined to explain the potent extent of this induction by peroxisome proliferators. As illustrated by this example, many regulatory and essential aspects of the peroxisome function remain to be discovered and further studies are needed to optimally exploit our knowledge on peroxisomes in order to treat human peroxisomal and related disorders. It is without saying that the range of human diseases and pathogenesis courses that might be addressed by these pathways is impressive. This is our opinion that some of these issues have been until now overlooked in terms of pharmacological development as more as disposition of peroxisomal acetyl-CoA might have currently specific unsuspected characteristics, depending on the cell type.

Conclusions and perspective

The impressive amount of data published on PPAR clearly indicate that in animals, PPARα receptor is a key element in the peroxisomal enzyme regulation by fatty acids, hormones and xenobiotics. Data illustrates that PPARα is controlled either positively or negatively by multiple ways. Attempts to explain large variations in peroxisomal enzymes gene expression level, especially in liver, by nutrients at the PPAR dependent level, should consider differences between species in the diet which in rodents is mainly based on carbohydrates except for the suckling period, and which in man consists of a mixed diet containing carbohydrates, fat and protids. In the latter dietary conditions (*i.e.* in the human subject), peroxisomal enzymes appear largely and constitutively expressed in contrast to rodents. This is not the case for mitochondrial fatty acid oxidation proteins which apparently keep inducible properties like in rodents. Attempts to correct human peroxisomal diseases are on progress using nutritional and pharmacological therapy through the ABC protein family encoding genes redundancy approach, but other pharmacological approaches are suggested, based on a better knowledge of the regulatory aspects of peroxisomal functions. The current topics of interest in peroxisome function and regulation might be the following: - restoration of impaired peroxisomal functions, - peroxisomes role in brain, - peroxisomes diversity, - peroxisomes in interaction with other organelles, - peroxisomes in development, and - unexplored peroxisome functions. In summary, our feeling is that interesting prospects could be made on peroxisome in relation with their involvement in particular developmental and physiological stages, and in new functions.

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References

1. **Roels F., Baes M., De Bie S**, *Peroxisomal disorders and regulation of genes*, Kluwer Academic Plenum Press, 2003, in press
2. **Issemann I., Green S.**, Activation of a member of the steroid hormone superfamily by peroxisome proliferators, *Nature*, **347**: 645-650, 1990
3. **Reddy J.K., Hashimoto T.**, Peroxisomal β-oxidation and Peroxisome Proliferator-Activated Receptors α: an adaptative metabolic system, *Ann. Rev. Nutr.,* **21**: 193-230, 2001
4. **Ferdinandusse S., Denis S., Mooijer P.A., Zhang Z., Reddy J.K., Spector A.A., Wanders R.J.**, Identification of the peroxisomal beta-oxidation enzymes involved in the biosynthesis of docosahexaenoic acid, *J. Lipid Res.,* **42**: 1987-1995, 2001
5. **Stolz D.B., Zamora R., Vodovotz Y., Loughran P.A., Billiar T.R., Kim Y.M., Simmons R.L., Watkins S.C.**, Peroxisomal localization of inducible nitric oxide synthase in hepatocytes, *Hepatology*, **36**: 81-93, 2002

6. **Chang C.C., South S., Warren D., Jones J., Moser A.B., Moser H.W., Gould S.J.**, Metabolic control of peroxisome abundance, *J. Cell Sci*., **112**: 1579-1590, 1999
7. **Latruffe N., Vamecq J.**, Evolutionary aspects of peroxisomes as cell organelles and genes encoding peroxisomal proteins, *Biol. Cell*, **92**: 389-395, 2000
8. **Petriv O.I., Pilgrim D.B., Rachubinski R.A., Titorenko V.I.**, RNA interference of peroxisome-related genes in C. elegans: a new model for human peroxisomal disorders, *Physiol. Genomics*, **10**: 79-91, 2002
9. **Berteaux-Lecellier V., Picard M., Thompson-Coffe C., Zickler D., Panvier-Adoutte A., Simonet J.M.**, A non-mammalian homolog of the PAF1 gene (Zellweger syndrome) discovered as a gene involved in caryogamy in the fungus Podospora anserina, *Cell*, **81**:1043-1051, 1995
10. **Liu L.X., Janvier K., Berteaux-Lecellier V., Cartier N., Benarous R., Aubourg P.,** Homo- and heterodimerization of peroxisomal ATP-binding cassette half-transporters, *J. Biol. Chem*., **274**: 32738-32743, 1999
11. **Duclos S., Bride J., Ramirez L.C., Bournot P.**, Peroxisome proliferation and beta-oxidation in Fao and MH1C1 rat hepatoma cells, HepG2 human hepatoblastoma cells and cultured human hepatocytes: effect of ciprofibrate, *Eur. J. Cell Biol*., **72**: 314-323, 1997
12. **El Kebbaj M.S., Malki M.C., Latruffe N.**, Properties of peroxisomes from jerboa (Jaculus orientalis), *Eur. J. Cell Biol*., **70**: 150-156, 1996
13. **Kabine M., Cherkaoui-Malki M., Clémencet M.-C., El Kebbaj M.S. and Latruffe N.**, Peroxisomal Changes During Hibernation of Jerboa (*Jaculus orientalis*), *J. Am. Oil Chem. Soc.,* **75**: 275-280, 1998
14. **Cimini A., Cristiano L., Bernardo A., Farioli-Vecchioli S., Stefanini S., Ceru M.P.**, Presence and inducibility of peroxisomes in a human glioblastoma cell line, *Biochim. Biophys. Acta*., **1474**: 397-409, 2000
15. **Kobayashi T, Shinnoh N, Kondo A, Yamada T**. Adrenoleukodystrophy protein-deficient mice represent abnormality of very long chain fatty acid metabolism, *Biochem. Biophys. Res. Commun.*, **232**: 631-636, 1997
16. **Fan C-Y., Pan J., Chu R., Lee D., Kluckman K.D., Usuda N., Singh I., Yeldandi A.V., Rao M.S., Maeda N., Reddy J.K.**, Hepatocellular and hepatic peroxisomal alterations in mice with a disrupted peroxisomal fatty acyl-coenzyme A oxidase gene, *J. Biol. Chem.*, **271**: 24689-24710, 1996
17. **Qi Q., Zhu Y., Pan J., Usuda N., Maeda N., Yeldandi A.V., Rao M.S., Hashimoto T., Reddy J.K**., Absence of spontaneous peroxisome proliferation in enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase-deficient mouse liver. Further support for the role of fatty acyl CoA oxidase in PPARα ligand metabolism, *J. Biol. Chem.*, **274**: 15775-15780, 1999
18. **Baes M., Huyghe S., Cameliet P., Declercq P.E., Collen D., Mannaerts G.P., Van Veldhoven P.P.** , Inactivation of the peroxisomal multifunctional protein-2 in mice impedes the degradation of not only 2-methyl-branched fatty acids and bile acid intermediates but also very long chain fatty acids, *J. Biol. Chem.*, **275**: 16329-16336, 2000
19. **Seedorf U., Raabe M., Ellinghaus P., Kannenberg F., Fobker M., Engel T., Denis S., Wouters F., Wirtz K.W., Wanders R.J.A**., Defective peroxisomal catabolism of branched fatty acyl coenzyme A in mice lacking the sterol carrier protein2/sterol carrier protein-x gene function, *Genes Dev.*, **12**: 1189-1201, 1998
20. **Lee S.S.T., Pineau T., Drago J., Lee E.J., Owens J.W., Kroetz D.I., Fernandez-Salguero P.M., Westphal H., Gonzalez F.J**., Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishement of the pleiotropic effect of peroxisome proliferators, *Mol. Cell. Biol.*, **15**: 312-322, 1995
21. **Michalik L, Desvergne B, Tan NS, Basu-Modak S, Escher P, Rieusset J, Peters JM, Kaya G, Gonzalez FJ, Zakany J, Metzger D, Chambon P, Duboule D, Wahli W.** Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice, *J. Cell Biol.*, **154**: 799-814, 2001
22. **Akiyama T.E., Sakai S., Lambert G., Nicol C.J., Matsusue K., Pimp S., Lee Y.H., Ricote M., Glass C.K., Brewer H.B., Gonzalez F.J.**, Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux, *Mol. Cell Biol.*, **22**: 2607-2619, 2002
23. **Baes M., Gressens P., Baumgart E., Carmeliet P., Casteels M., Fransen M., Evrard P., Fahimi D., Declercq P, Collen D**., et al, A mouse model for Zellweger syndrome, *Nature Genet.,* **17**: 49-56, 1997
24. **Faust PL, Hatten ME.** Targeted deletion of the PEX2 peroxisome assembly gene in mice provides a model for Zellweger syndrome, a human neuronal migration disorder, *J. Cell Biol*., **139**: 1293-1305, 1997
25. **Baes M, Dewerchin M, Janssen A, Collen D, Carmeliet P**. Generation of Pex5-loxP mice allowing the conditional elimination of peroxisomes, *Genesis*, **32**: 177-178, 2002
26. **Bjorkman J, Tonks I, Maxwell MA, Paterson C, Kay GF, Crane DI**. Conditional inactivation of the peroxisome biogenesis Pex13 gene by Cre-loxP excision, *Genesis*, **32**: 179-80, 2002
27. **Nilakatan V., LI X., Glauert H.P., Spear B.T.**, Increased liver-specific catalase activty in transgenic mice, *DNA and Cell Biology*, **15**: 625-630, 1996
28. **Kiema T.R., Taskinen J.P., Pirila P.L., Koivuranta K.T., Wierenga R.K., Hiltunen J.K.**, Organization of the multifunctional enzyme type 1: interaction between N- and C-terminal domains is required for the hydratase-1/isomerase activity, *Biochem. J*., **367**: 433-441, 2002
29. **Cherkaoui-Malki M., Meyer K., Cao W.Q., Latruffe N., Yeldandi A.V., Rao M.S., Bradfield C.A., Reddy J.K.**, Identification of novel peroxisome proliferator-activated receptor alpha (PPARalpha) target genes in mouse liver using cDNA microarray analysis, *Gene Expr*., **9**: 291-304, 2001
30. **Zipper J.**, Proliferation of myocardial peroxisomes caused by several agents and conditions, *J. Mol. Cell Cardiol*., **29**: 149-161, 1997
31. **Fan C.Y., Pan L., Usuda N., Yeldandi A.V., Rao M.S., Reddy J.K**., Steatohepatitis, spontaneaous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase, *J. Biol. Chem.,* **273**: 15639-15645, 1998

32. **Tobin K.A., Steineger H.H., Alberti S., Spydevold O., Auwerx J., Gustafsson J.A., Nebb H.I.**, Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-alpha, *Mol. Endocrinol.,* **14**: 741-752, 2000
33. **Chao P.M., Chao C.Y., Lin F.J., Huang C.**, Oxidized frying oil up-regulates hepatic acyl-CoA oxidase and cytochrome P450 4 A1 genes in rats and activates PPARalpha, *J. Nutr.,* **131**: 3166-3174, 2001
34. **Moya-Camarena S.Y., Vanden Heuvel J.P., Blanchard S.G., Leesnitzer L.A., Belury M.A.**, Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha, *J. Lipid Res.,* **40**: 1426-1433, 1999
35. **Zomer A.W., van Der Burg B., Jansen G.A., Wanders R.J., Poll-The B.T., van Der Saag P.T.,** Pristanic acid and phytanic acid: naturally occurring ligands for the nuclear receptor peroxisome proliferator-activated receptor alpha, *J. Lipid Res*., **41**: 1801-1807, 2000
36. **Vamecq J., Latruffe N.**, Medical significance of peroxisome proliferator-activated receptors, *Lancet*, **354**(9173): 141-148, 1999
37. **Kabine M., , Clémencet M.-C., El Kebbaj M.S. and Latruffe N, Cherkaoui-Malki M.** Changes of peroxisomal fatty acid metabolism during cold acclimatization in hibernating Jerboa (jaculus orientalis), *Biochimie* (in press), 2003
38. **Goglia F., Liverini G., Lanni A., Iossa S., Barletta A.,** Morphological and functional modifications of rat liver peroxisomal subpopulations during cold expusure, *Exp. Biol.,* **48**: 127-133, 1989
39. **Xia T., Mostafa N., Bhat B.G., Florant G.L., Coleman R.A.,** Selective retention of essential fatty acids : the role of hepatic monoacylglycerol acyltranferase, *Am. J. Physiol*., **265**: 414-419, 1993
40. **Nedergaard J., Alexon S., Cannon B.,** Cold adaptation in the rat: increased brown fat peroxisomal β-oxidation related to maximal mitochondrial oxidative capacity, *Am. J. Physiol.,* **239**: C208-C216, 1980
41. **Guardiola-Diaz H.M., Rehnmark S., Usuda N., Albrektsen T., Feltkamp D., Gustafsson J.A., Alexson S.E.**, Rat peroxisome proliferator-activated receptors and brown adipose tissue function during cold acclimatization, *J. Biol. Chem.,* **274**: 23368-23377, 1999
42. **Yu S., Cao W-Q., Kashireddy P., Meyer K., Jia Y., Hughes D.E., Tan Y., Feng J., Yeldandi A.V., Rao M.S., Costa R.H., Gonzalez F.J., Reddy J.K.**, Human peroxisome proliferator-activated receptor α (PPARα) supports the induction of peroxisome proliferation in PPARa-deficient mouse liver, *J. Biol. Chem.*, **276**: 42485-42491, 2001
43. **Hsu M.H., Savas U., Griffin K.J., Johnson E.F.**, Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor alpha in HepG2 cells, *J. Biol. Chem.,* **276**: 27950-27958, 2001
44. **Lawrence J.W., Li Y., Chen S., DeLuca J.G., Berger J.P., Umbenhauer D.R., Moller D.E., Zhou G.**, Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) alpha. PPAR alpha fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expresson, *J. Biol. Chem.,* **276**: 31521-31527, 2001
45. **Mascaro C., Acosta E., Ortiz J.A., Marrero P.F., Hegardt F.G., Haro D.**, Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor, *J. Biol. Chem.,* **273**: 8560-8563, 1998
46. **Zhang X., Chen L., Hardwick J.P.**, Promoter activity and regulation of the CYP4F2 leukotriene B(4) omega-hydroxylase gene by peroxisomal proliferators and retinoic acid in HepG2 cells, *Arch. Biochem. Biophys.,* **378**: 364-376, 2000
47. **Chu R., Madison L.D., Lin Y., Kopp P., Rao M.S., Jameson J.L., Reddy J.K.**, 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, 1995
48. **Miyata K.S., McCaw S.E., Patel H.V., Rachubinski R.A., Capone J.P.**, The orphan nuclear hormone receptor LXR alpha interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling, *J. Biol. Chem.,* **271**: 9189-9192, 1996
49. **Sinal C.J., Yoon M., Gonzalez F.J.,** Antagonism of the actions of peroxisome proliferator-activated receptor-alpha by bile acids, *J. Biol. Chem.,* **276**: 47154-47162, 2001
50. **Kassam A., Winrow C.J., Fernandez-Rachubinski F., Capone J.P., Rachubinski R.A.**, The peroxisome proliferator response element of the gene encoding the peroxisomal beta-oxidation enzyme enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase is a target for constitutive androstane receptor beta/9-cis-retinoic acid receptor-mediated transactivation, *J. Biol. Chem.,* **275**: 4345-4350, 2000
51. **Shy Y., Hon M., Evans R.M.**, The peroxisome proliferator-activated receptor delta, an integrator of transcriptional repression and nuclear receptor signaling, *Proc. Natl. Acad. Sci. USA,* **99**: 2613-2618, 2002
52. **Kassam A., Capone J.P., Rachubinski R.A.**, The short heterodimer partner receptor differentially modulates peroxisome proliferator-activated receptor alpha-mediated transcription from the peroxisome proliferator-response elements of the genes encoding the peroxisomal beta-oxidation enzymes acyl-CoA oxidase and hydratase-dehydrogenase, *Mol. Cell Endocrinol*., **176**: 49-56, 2001
53. **Zhou Y.C., Davey H.W., McLachlan M.J., Xie T., Waxman D.J.**, Elevated basal expression of liver peroxisomal beta-oxidation enzymes and CYP4A microsomal fatty acid omega-hydroxylase in STAT5b(-/-) mice: cross-talk *in vivo* between peroxisome proliferator-activated receptor and signal transducer and activator of transcription signaling pathways, *Toxicol. Appl. Pharmacol*., **182**: 1-10, 2002
54. **Miyata K.S., McCaw S.E., Meertens L.M., Patel H.V., Rachubinski R.A., Capone J.P.**, Receptor-interacting protein 140 interacts with and inhibits transactivation by, peroxisome proliferator-activated receptor alpha and liver-X-receptor alpha, *Mol. Cell Endocrinol*., **146**: 69-76, 1998
55. **Mochizuki K., Suruga K., Sakaguchi N., Takase S., Goda T.**, Major intestinal coactivator p300 strongly activates peroxisome proliferator-activated receptor in intestinal cell line, Caco-2, *Gene*, **291**: 271-277, 2002

56. **Surapureddi S., Yu S., Bu H., Hashimoto T., Yeldandi A.V., Kashireddy P., Cherkaoui-Malki M., Qi C., Zhu Y.J., Rao M.S., Reddy J.K.**, Identification of a transcriptionally active peroxisome proliferator-activated receptor alpha -interacting cofactor complex in rat liver and characterization of PRIC285 as a coactivator, *Proc. Natl. Acad. Sci. USA,* **99**: 11836-11841, 2002
57. **Latruffe N., Cherkaoui-Malki M., Nicolas-Frances V., Clemencet M.C., Jannin B., Berlot J.P.**, Regulation of the peroxisomal beta-oxidation-dependent pathway by peroxisome proliferator-activated receptor alpha and kinases, *Biochem Pharmacol.,* **60**: 1027-1032, 2000
58. **Lazennec G., Canaple L., Saugy D., Wahli W.**, Activation of peroxisome proliferator-activated receptors (PPARs) by their ligands and protein kinase A activators, *Mol. Endocrinol*., **14**: 1962-1975, 2000
59. **Barger P.M., Browning A.C., Garner A.N., Kelly DP.**, p38 mitogen-activated protein kinase activates peroxisome proliferator-activated receptor alpha: a potential role in the cardiac metabolic stress response, *J. Biol. Chem*., **276**: 44495-44501, 2001
60. **Beier K., Volkl A., Fahimi H.D.**, TNF-alpha downregulates the peroxisome proliferator activated receptor-alpha and the mRNAs encoding peroxisomal proteins in rat liver, *FEBS Lett*., **412**: 385-387, 1997
61. **Meertens L.M., Miyata K.S., Cechetto J.D., Rachubinski R.A., Capone J.P.**, A mitochondrial ketogenic enzyme regulates its gene expression by association with the nuclear hormone receptor PPARalpha, *EMBO J*., **17**: 6972-6978, 1998
62. **Juge-Aubry C.E., Kuenzli S., Sanchez J.C., Hochstrasser D., Meier C.A.**, Peroxisomal bifunctional enzyme binds and activates the activation function-1 region of the peroxisome proliferator-activated receptor alpha, *Biochem J*., **353**: 253-258, 2001
63. **Tan N.S., Shaw N.S., Vinkenbosch N., Yasmin R., Desvergne B., Walhi W., Noy N.**, Selective cooperation between fatty acid binding protein and peroxisome proliferator-activated receptor in regulating transcription, *Mol. Cell Biol.,* **22**: 5114-5127, 2002, and *Mol. Cell Biol.,* **22**: 6318, 2002
64. **Powers J.M., Moser H.W**., Peroxisomal disorders: genotype, phenotype, major neuropathologic lesions, and pathogenesis, *Brain Pathol*., **8**: 101-120, 1998
65. **Wanders R.J.A., Vreken P., Ferdinandusse S., Janssen G.A., Waterham H.R., Van Roermund C.W.T. and Van Grunsven E.G.** Peroxisomal fatty acid α- and β-oxidation in humans: enzymology, peroxisomal metabolite transporters and peroxisomal diseases, *Biochem. Soc. Trans.,* **29**(Part 2): 250-267, 2001
66. **Fourcade S.,Savary S. Gondcaille C., Berger J., Netik A., Cadepond F., El Etr M., Molzer B., Bugaut M.** Thyroid hormone induction of the ABCD2 gene: prospect of a therapy for X-linked adrenoleukodystrophy, *Mol. Pharmacol.*, **63**: 1296-1303, 2003
67. **Caira F., Clemencet M-C., Cherkaoui-Malki M., Dieuaide-Noubhani M., Pacot C., Van Veldhoven P.P., Latruffe N.**, Differential regulation by a peroxisome proliferator of the different multifunctional proteins in guinea pig: cDNA cloning of the guinea pig D-specific multifunctional protein 2, *Biochem. J*., **330**: 1361-1368, 1998
68. **Motojima K., Passilly P., Peters J.M., Gonzalez F.J., Latruffe N.**, Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue - and inducer-specific manner, *J. Biol. Chem*., **273**: 16710-16714, 1998
69. **Le Jossic-Corcos C., Bournot P.**, Regulation of farnesyl pyrophosphate synthase gene expression by fatty acids in"Peroxisomal Disorders and Regulation of Genes" (Roels F., De Baes M., De Bie S eds) Kluwer Academic Plenum press (in preparation), 2003
70. **Nicolas-Frances V., Dasari V.K., Abruzzi E., Osumi T., Latruffe N.**, The peroxisome proliferator response element (PPRE) present at positions -681/-669 in the rat liver 3-ketoacyl-CoA thiolase B gene functionally interacts differently with PPARalpha and HNF-4*, Biochem. Biophys. Res. Commun.,* **269**: 347-351, 2000