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PEROXISOMAL LIPID METABOLISM

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INTRODUCTION

Peroxisomes are cell organelles present in virtually all eukaryotic cells. According to de Duve's classical definition, the organelles contain one or more hydrogen peroxide–producing flavin oxidases together with catalase that de-

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composes hydrogen peroxide to molecular oxygen and water. The name peroxisome was coined to emphasize the organelle's role in hydrogen peroxide metabolism (11). In addition to oxidases and catalase, peroxisomes harbor a variety of other enzymes. At present, more than 50 enzymes have been described in mammalian peroxisomes, more than half of which play a role in lipid metabolism (58, 99, 106). Thus mammalian peroxisomal enzymes are involved in the degradation of fatty acids and fatty acid derivatives via β-oxidation; the β-oxidation of the side chain of cholesterol, which results in the formation of bile acids; the synthesis of ether glycerolipids, cholesterol, and dolichols; and fatty acid elongation. Functions not directly related to lipid metabolism are catabolism of purines and polyamines, metabolism of amino acids and glyoxylate, and inactivation of reactive oxygen species such as hydrogen peroxide, superoxide anions, and epoxides (58, 107, 122). A surprising feature of the peroxisomal lipid-metabolizing enzymes in mammalian cells is enzyme duplication in other cell compartments (58). For example, β-oxidation enzymes are present not only in peroxisomes but also in mitochondria and enzymes involved in cholesterol. Dolichol synthesis also occurs in the endoplasmic reticulum and, in some cases, in the cytosol.

Glyoxysomes and glycosomes are the other members of the peroxisome family (69, 99). They host a number of enzymes typical of peroxisomes, such as the β-oxidation enzymes. Additionally, glyoxysomes possess some or all of the glyoxylate cycle enzymes, which in conjunction with the glyoxysomal β-oxidation enzymes enable the glyoxysome to convert fat into sugar. Glyoxysomes are found in oil-rich plant seeds and in certain yeasts grown on fatty acids or alkanes, whereas glycosomes, which contain part of the glycolytic sequence, are found in trypanosomatids.

The importance of peroxisomes in mammalian metabolism is underscored by the discovery of carcinogenic peroxisome proliferators (57, 75, 77, 79) and by the occurrence of peroxisomal diseases (24, 117). Treating rodents with a high-fat diet or with a number of structurally diverse chemicals termed peroxisome proliferators (79), which include the hypolipidemic fibric acid derivatives, results in the proliferation of peroxisomes in liver and (to a lesser extent) in other tissues, such as kidney, intestinal mucosa, and heart (57, 67, 68). Peroxisome proliferation is characterized by an increase in peroxisome number and fractional volume and is accompanied by the induction of some (e.g. the β-oxidation enzymes) but not all peroxisomal enzymes. Other species, such as the guinea pig and primates (including humans), are much less or not at all responsive to treatment with peroxisome proliferators (57, 80). Sustained induction of peroxisome proliferation and an increase in peroxisomal fatty acid β-oxidation in response to peroxisome proliferators result in greater generation of hydrogen peroxide in the livers of rats and mice (12, 80). Despite the fact that these agents fail to damage DNA directly, rats and mice chronically

exposed to these agents develop liver tumors (75, 80, 119), which has led investigators to postulate that secondary events associated with or resulting from the stimulation of genes that encode the fatty acid β-oxidation system may play a role in the neoplastic process (75, 77, 79, 80).

The recognition in humans of inheritable diseases caused by deficiency of peroxisomes or single peroxisomal enzymes has provided valuable insight into the metabolic functions of these organelles and the gene products involved in peroxisome assembly (24, 117). The prototype of this group of inherited diseases is the Zellweger syndrome, which is characterized by a lack of functional peroxisomes as a result of a presumed defect in peroxisome biogenesis. Zellweger patients suffer from severe functional and anatomical deficits and usually die within the first year of life (24).

This review is limited to a discussion of mammalian peroxisomes. In particular, we emphasize the role of mammalian peroxisomes in lipid metabolism, the phenomenon of peroxisome proliferation and the mechanism of peroxisomal enzyme induction, and the disorders of peroxisomal lipid metabolism.

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PEROXISOMAL β-OXIDATION

In lower eukaryotes such as yeasts and fungi, peroxisomes appear to be the only subcellular site of β-oxidation (99, 106). Plant peroxisomes can also β-oxidize fatty acids (10), but whether plant mitochondria contain a functional β-oxidation system remains controversial. In animal cells, peroxisomes as well as mitochondria degrade fatty acids via β-oxidation (51, 52). In mammals, substrates for β-oxidation include short- ($< C_8$), medium- (C_{8-C12}), long- (C_{14-C20}), and very long-chain ($> C_{20}$) fatty acids, dicarboxylic fatty acids, isoprenoid-derived 2-methyl-branched fatty acids (e.g. pristanic acid), the side chain of the bile acid intermediates di- and trihydroxycoprostanic acids (side chain of cholesterol during bile acid synthesis), and the side chains of prostaglandins and other eicosanoids, fat-soluble vitamins, and certain xenobiotics.

Fatty Acid Activation and Penetration into the Peroxisome

Before a fatty acid can be degraded via β-oxidation, it must first be activated to its CoA derivative. Peroxisomal membranes contain at least two acyl-CoA synthetases: a long-chain acyl-CoA synthetase, which activates long-chain fatty acids (59, 87), and a very long-chain acyl-CoA synthetase, which activates very long-chain fatty acids (54, 88). The catalytic site of long-chain acyl-CoA synthetase is exposed to the cytosol (48, 54, 59). The exact location (cytosolic or metrical side of the membrane) of the catalytic site of the very long-chain acyl-CoA synthetase remains controversial (48, 54). Long-chain acyl-CoA synthetases are also found in the mitochondrial outer membrane and in the endoplasmic reticulum (59, 87). Peroxisomal and extraperoxisomal synthetases

display the same molecular, kinetic, and immunochemical characteristics, which suggests a high degree of homology (29). Very long-chain acyl-CoA synthetase is also present in the endoplasmic reticulum but is absent from mitochondria (88), which may explain why very long-chain fatty acids are oxidized predominantly in peroxisomes (see below). Isoprenoid-derived branched fatty acids can be activated by peroxisomes, mitochondria, and endoplasmic reticulum (112, 118), possibly via the long-chain acyl-CoA synthetases present in these cell compartments. Preliminary evidence that peroxisomes contain a separate branched-chain acyl-CoA synthetase awaits further confirmation (72). Short and medium straight-chain fatty acids are activated in the mitochondrial matrix (91); medium-chain fatty acids can also be activated to a certain extent by long-chain acyl-CoA synthetases present in the mitochondrial outer membrane and in the peroxisomal and endoplasmic reticulum membranes. Dicarboxylic fatty acids (105), prostaglandins (83), and the bile acid intermediates di- and trihydroxycoprostanic acids (84a) are activated solely in the endoplasmic reticulum. The bile acid intermediates are activated by a separate enzyme, trihydroxycoprostanoyl-CoA synthetase, that is present only in liver (122).

The fact that dicarboxylic fatty acids and prostaglandins are not activated by mitochondria or peroxisomes suggests that these molecules are not substrates for long-chain acyl-CoA synthetases but rather for a specific enzyme(s). Unlike in mitochondria, long-chain acyl-CoAs (and other CoA esters) do not need carnitine for their penetration into the peroxisome (60, 97). The peroxisomal membrane does not contain the carnitine palmitoyltransferase/carnitine translocase complex required for transport of acyl-CoA across the mitochondrial inner membrane. Isolated peroxisomes and peroxisomes in permeabilized hepatocytes are permeable to small, water-soluble molecules, including a variety of substrates and cofactors for peroxisomal enzymes (108). The nonspecific permeability of the peroxisomal membrane appears to result from the presence of hydrophilic proteinaceous pores in the membrane (108). Whether acyl-CoAs, which are amphiphilic and tend to accumulate in the lipid phase of membranes, also diffuse via the pores or penetrate via another mechanism remains unknown.

Peroxisomal β-Oxidation Enzymes

As does mitochondrial β-oxidation, peroxisomal β-oxidation proceeds via four consecutive reactions that take place in the peroxisomal matrix: a first oxidation reaction, in which the acyl-CoA is desaturated to a 2-*trans*-enoyl-CoA; a hydration reaction, which converts the unsaturated intermediate to a L-3-hydroxyacyl-CoA; a second oxidation step, which dehydrogenates the hydroxy intermediate to a 3-ketoacyl-CoA; and finally, a thiolytic cleavage, which releases acetyl-CoA and an acyl-CoA two carbon atoms shorter than the

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original molecule that can re-enter the spiral for the next round of β-oxidation (29, 52).

Despite this similarity in reaction mechanism, the mitochondrial and peroxisomal β-oxidation systems differ in several respects. For example, the reactions in peroxisomes and mitochondria are catalyzed by different proteins. The first reaction of peroxisomal β-oxidation (desaturation) is catalyzed by a FAD-containing oxidase that donates electrons directly to molecular oxygen, thereby producing hydrogen peroxide that is disposed of by catalase. Rat liver peroxisomes contain three acyl-CoA oxidases (84a, 109, 110): (*a*) palmitoyl-CoA oxidase, which oxidizes the CoA esters of medium-, long-, and very long-chain fatty acids; medium- and long-chain dicarboxylic fatty acids and prostaglandins; (*b*) pristanoyl-CoA oxidase, which oxidizes the CoA esters of 2-methyl-branched fatty acids such as pristanic acid but which also exhibits some activity with the CoA esters of straight-chain and dicarboxylic fatty acids; and (*c*) trihydroxycoprostanoyl-CoA oxidase, which oxidizes the CoA esters of the bile acid intermediates di- and trihydroxycoprostanic acids. Palmitoyl-CoA oxidase has a molecular mass of 150 kDa and consists of subunits of 72, 52, and 21 kDa (29). The smaller subunits are formed in vivo by proteolytical cleavage of the larger subunit. The enzyme is induced by treating the rats with peroxisome proliferators (29, 67, 68, 78, 84a, 109). Palmitoyl-CoA oxidase is present in liver as well as in extrahepatic tissues. Unlike palmitoyl-CoA oxidase, pristanoyl-CoA oxidase is not inducible, but like palmitoyl-CoA oxidase, it is present in liver and in extrahepatic tissues. Pristanoyl-CoA oxidase has a molecular mass of 420 kDa and consists of identical subunits of 70 kDa (84a, 109). Trihydroxycoprostanoyl-CoA oxidase is not inducible and is present only in liver. It has a molecular mass of 139 kDa and consists of identical subunits of 69 kDa (84a, 109).

Human peroxisomes contain only two acyl-CoA oxidases that are present in liver as well as in extrahepatic tissues (113). The first enzyme, palmitoyl-CoA oxidase, has a molecular mass and subunit composition similar to that of rat palmitoyl-CoA oxidase, cross-reacts with polyclonal antibodies raised against the rat enzyme and, as does the rat enzyme, oxidizes the CoA esters of straight-chain and dicarboxylic fatty acids as well as those of prostaglandins (113). The second human enzyme, branched-chain acyl-CoA oxidase, is a monomeric 70 kDa protein that oxidizes the CoA esters of 2-methyl-branched fatty acids as well as those of the bile acid intermediates di- and trihydroxycoprostanic acids, which also possess a 2-methyl substitution in their side chain. This enzyme exhibits minimal activity with the CoA esters of straight-chain fatty acids. It does not cross-react with polyclonal antibodies raised against rat palmitoyl-CoA oxidase or against rat pristanoyl-CoA oxidase, which suggests that it may be related to rat trihydroxycoprostanoyl-CoA oxidase (113). Cloning and sequencing the members of the oxidase family will

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confirm or refute this assertion. The second (hydration) and third (NAD^+-dependent dehydrogenation) steps of peroxisomal β-oxidation are catalyzed by a single multifunctional protein that also displays δ^3, δ^2-enoyl-CoA isomerase activity required for the oxidation of unsaturated fatty acids (73) (see below). The rat enzyme, which is inducible by treatment with peroxisome proliferators, is a 78 kDa monomeric protein. Its human counterpart has a similar molecular mass and cross-reacts with antisera raised against the rat enzyme (81).

The final step in peroxisomal β-oxidation is catalyzed by a 3-ketoacyl-CoA thiolase. Two peroxisomal thiolase genes, each of which encodes a slightly different amino acid sequence, have been detected in rat liver (32). One of the genes is activated by treatment with peroxisome proliferators; the other is activated only slightly or not at all. The inducible enzyme consists of two identical 41 kDa subunits synthesized as 44 kDa precursors containing a cleavable amino-terminal presequence, which is split off following import of the enzyme into the peroxisome. Because the noninducible enzyme has not yet been identified, whether the two enzymes differ in substrate specificity or kinetic properties remains unknown. Human tissues contain only one thiolase gene (5). Human thiolase and its precursor have the same molecular masses as the inducible rat enzyme and its precursor and cross-react with polyclonal antibodies raised against the rat enzyme (117). Excluding noninducible thiolase, all mentioned rat enzymes have been purified (70, 71, 84a, 109). Their deduced amino acid sequences are known, with the exception of pristanoyl-CoA oxidase and trihydroxycoprostanoyl-CoA oxidase (32, 70, 71). Among the human enzymes, only palmitoyl-CoA oxidase, the branched-chain acyl-CoA oxidase, and the bifunctional enzyme have been purified (81, 113). The deduced amino acid sequence is known for palmitoyl-CoA oxidase (114) and thiolase (5).

Peroxisomes (and mitochondria) also contain the auxiliary enzymes required for the oxidation of unsaturated fatty acids: 2,4-dienoyl-CoA reductase and δ^3, δ^2-enoyl-CoA isomerase (33). During the β-oxidation of fatty acids that have a double bond extending from an even-numbered carbon atom, a 2,4-dienoyl-CoA intermediate is formed. The dienoyl-CoA is not further degraded, but it is first reduced to a 3-*trans*-enoyl-CoA by 2,4-dienoyl-CoA reductase, an NADPH-dependent enzyme. The 3-*trans*-enoyl-CoA is then isomerized to a 2-*trans*-enoyl-CoA by the δ^3, δ^2-enoyl-CoA isomerase (73). The 2-*trans*-enoyl-CoA is further degraded via the classical β-oxidation pathway. The peroxisomal 2,4-dienoyl-CoA reductase has not yet been purified. The two mitochondrial dienoyl-CoA reductases are induced by treating rodents with peroxisome proliferators (28). Whether the peroxisomal enzyme is also inducible remains to be investigated. As mentioned above, the peroxisomal δ^3, δ^2-isomerase activity is catalyzed by the inducible multifunctional protein. Whether a second, separate isomerase exists in peroxisomes is still under

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debate (100). The isomerase(s) may also be involved in the oxidation of fatty acids that have a double bond extending from an odd-numbered carbon atom. During the β-oxidation of these fatty acids, a 3-enoyl-CoA is formed that must be isomerized to a 2-enoyl-CoA before β-oxidation can proceed. Recently, however, this view was challenged. Evidence indicates that β-oxidation of odd-numbered unsaturated fatty acids stops as soon as a 5-enoyl-CoA intermediate is formed (102). The 5-enoyl-CoA is then reduced to a saturated acyl-CoA by a putative NADPH-dependent 5-enoyl-CoA reductase. The enzyme has not yet been identified. Finally, peroxisomes also contain a 2-enoyl-CoA hydratase 2 that reversibly converts a 2-*trans*-enoyl-CoA into a D-3-hydroxyacyl-CoA (34). Because the D-3-hydroxy stereoisomer is not a substrate for the 3-hydroxyacyl-CoA dehydrogenase reaction catalyzed by the multifunctional protein, the function of the hydratase 2 remains unclear.

Differences between Peroxisomal and Mitochondrial Fatty Acid Oxidation

We have mentioned that although peroxisomes and mitochondria catalyze similar reactions, the enzymes in each system are different proteins. The second important difference between the mitochondrial and peroxisomal systems is that peroxisomal β-oxidation is not directly coupled to an electron transfer chain that conserves energy by means of oxidative phosphorylation (51, 60). The energy released in the first oxidation step of peroxisomal β-oxidation is H_2O_2, which is lost as heat. In contrast, the energy produced in the second oxidation step (NAD^+ reduction) is conserved in the form of the high energy level electrons of NADH. Because isolated peroxisomes are permeable to NADH, NADH formed in the peroxisome can probably freely diffuse into the cytosol. Thus, compared with mitochondrial β-oxidation, peroxisomal β-oxidation is only approximately half as efficient in conserving energy. Because it generates heat, peroxisomal β-oxidation has been thought to play a role in thermogenesis (53, 99).

Peroxisomal β-oxidation is unusual in that it does not go to completion but rather catalyzes a limited number of β-oxidation cycles and hence acts as a chain-shortening system (52, 97). The number of cycles catalyzed in the intact cell may depend on the chain length of the substrate, but what halts peroxisomal β-oxidation remains uncertain. The peroxisomal β-oxidation enzymes are only slightly active toward short-chain acyl-CoAs (52, 110, 113). Other possible reasons for this interruption include product inhibition by accumulating intermediates and the presence in the peroxisomal matrix of acyl-CoA hydrolases (3) and carnitine octanoyltransferase (17), which may compete for the shortened acyl-CoAs.

Another difference between mitochondrial and peroxisomal β-oxidation is the fate of the metabolic products. Acetyl-CoA generated by mitochondrial

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β-oxidation enters the Krebs cycle for further oxidation or condenses to ketone bodies (liver) that serve as oxidizable substrates for extrahepatic tissues (62). Peroxisomes, on the other hand, lack Krebs cycle enzymes and ketogenic enzymes (60). They produce acetyl-CoA, acetate, acetylcarnitine, and acetoacetyl-CoA (36, 97). The latter three products are likely generated by acetyl-CoA hydrolase (17), carnitine acetyltransferase (3), and thiolase, respectively. All three enzymes are present in the peroxisomal matrix. The fate of the acetyl units produced by peroxisomes is unknown. They may be further oxidized in the mitochondria or used for biosynthetic purposes (e.g. fatty acid synthesis, fatty acid elongation, or cholesterol and dolichol synthesis). The first β-oxidation cycle of 2-methyl-branched compounds (2-methyl-branched fatty acids and the bile acid intermediates di- and trihydroxycoprostanic acids) releases propionyl-CoA instead of acetyl-CoA. Studies in isolated hepatocytes have shown that a major portion of the propionyl-CoA is oxidized to CO_2 (71, 112), which indicates that it had entered the mitochondria, where it was oxidized in the Krebs cycle after conversion to succinyl-CoA via methylmalonyl-CoA. Part of the succinyl-CoA formed may also be used for gluconeogenesis. The shortened acyl-CoAs produced in peroxisomes can either be oxidized in the mitochondria (medium- and long-chain acyl-CoAs) or esterified to glycerolipids in the endoplasmic reticulum (long-chain acyl-CoAs).

Substrates for Mitochondrial and Peroxisomal β-Oxidation

The substrate spectra of mitochondria and peroxisomes partly overlap. Isolated mitochondria as well as isolated peroxisomes are capable of β-oxidizing medium- and long-chain mono- and dicarboxylic fatty acids, 2-methyl-branched fatty acids, and certain eicosanoids, such as prostaglandins. Bile acid intermediates and very long-chain fatty acids are oxidized by peroxisomes and only poorly (very long-chain fatty acids) or not at all (bile acid intermediates) by mitochondria. Conversely, short-chain fatty acids are poorly or not at all oxidized by peroxisomes, but they are readily degraded in mitochondria. The contribution of mitochondria and peroxisomes to substrate oxidation in the intact cell depends not only on the capability of each organelle to oxidize a particular substrate, but also on the substrate affinity, the specific β-oxidizing activity, and the relative abundance of each organelle.

In the intact cell or organism, mitochondria oxidize the major portion of the medium- and long-chain fatty acids (18, 60, 97). Long-chain fatty acids constitute the bulk of fatty acids in the organism. Their abundance makes them the only significant source of metabolic fuel among the β-oxidation substrates. The dominant role of mitochondria in the oxidation of long-chain fatty acids seems a logical consequence of the fact that mitochondrial β-oxidation conserves almost double the energy that peroxisomes do.

Very long-chain fatty acids are oxidized predominantly, if not exclusively,

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by peroxisomes (52, 74) because mitochondria lack very long-chain acyl-CoA synthetase (40, 54). In humans, very long-chain acyl-CoAs are oxidized almost exclusively via palmitoyl-CoA oxidase (113), and in the rat, they are oxidized partially via palmitoyl-CoA oxidase and partially via pristanoyl-CoA oxidase (110). Very long-chain fatty acids are shortened in peroxisomes to long-chain fatty acids that can be further oxidized in the mitochondria.

Dicarboxylic fatty acids are formed via ω-oxidation of long-chain monocarboxylic fatty acids under conditions of fatty acid overload (e.g. uncontrolled diabetes) and in situations in which mitochondrial fatty acid oxidation is impaired (e.g. inherited defects, inhibitors). The first step in ω-oxidation is ω-hydroxylation of fatty acid in the endoplasmic reticulum catalyzed by lauric acid ω-hydroxylase, a member of the cytochrome $_P$450IVA subfamily induced by peroxisome proliferators (23, 57). The ω-hydroxy fatty acid is then dehydrogenated to a dicarboxylic acid in the cytosol and converted to its CoA derivative by an acyl-CoA synthetase present in the endoplasmic reticulum (105). Most evidence points to a dominant role for peroxisomes in dicarboxylyl-CoA oxidation (8, 56). The long-chain dicarboxylic acids are shortened in peroxisomes to more polar medium-chain dicarboxylic acids that can be excreted in urine. In humans, dicarboxylyl-CoAs are oxidized almost exclusively via palmitoyl-CoA oxidase (113). A significant portion also appears to be oxidized via pristanoyl-CoA oxidase (110) in the rat.

Pristanic acid (2,6,10,14-tetramethylpentadecanoic acid), a 2-methyl-branched, isoprenoid-derived fatty acid that originates from the oxidative decarboxylation of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), accumulates in tissues and body fluids of patients with peroxisome deficiency disorders. It is minimally or not at all oxidized by fibroblasts from such patients (111, 117). Animal experiments also indicate that peroxisomes play a dominant role in the oxidation of 2-methyl-branched fatty acids (112). Pristanic acid is activated in peroxisomes, mitochondria, and endoplasmic reticulum, possibly by the long-chain acyl-CoA synthetases (112, 118). In peroxisomes, pristanoyl-CoA is oxidized via pristanoyl-CoA oxidase in the rat (110) and via the branched chain acyl-CoA oxidase in humans (113).

Other compounds that are excreted in the urine as metabolites shortened via β-oxidation are eicosanoids such as prostaglandins, thromboxanes, prostacyclin, leukotrienes, and the fat-soluble vitamins E and K. In vitro experiments on animal tissues (13, 41, 83) and the observation of urinary metabolite patterns in patients with peroxisome deficiency disorders (13, 61) have established the key involvement of peroxisomes in the degradation of prostaglandins and leukotrienes B_4 and E_4. The results of these experiments suggest that other eicosanoids, such as thromboxanes and prostacyclin, are also degraded by peroxisomes. Prostaglandins are activated to their CoA derivatives in the endoplasmic reticulum (83) and are oxidized via palmitoyl-CoA oxidase in

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rats and humans (110, 113). Because of the predominant role of peroxisomes in the oxidation of 2-methyl-branched fatty acids (112), one can speculate that these organelles are also responsible for the degradation of the side chains of vitamins E and K.

The liver is the only organ that can degrade cholesterol. In the first series of reactions, the steroid nucleus is modified (reduction of the double bond, hydroxylations), and one of the terminal methyl groups of the side chain is oxidized, which gives rise to the C_{27} bile acid intermediates di- and trihydroxycoprostanic acids. The C_{27} bile acids are then activated in the endoplasmic reticulum by trihydroxycoprostanoyl-CoA synthetase (84a). The activated side chains of di- and trihydroxycoprostanic acid are shortened by one round of peroxisomal β-oxidation, thereby yielding the CoA esters of chenodeoxycholic and cholic acid, respectively (43). The CoA esters of these primary bile acids are then conjugated with taurine or glycine. These reactions take place in the peroxisome as well as in the endoplasmic reticulum (44) and the products are excreted in bile. The side chains of the bile acid intermediates are oxidized via trihydroxycoprostanoyl-CoA oxidase in the rat (110) and via the branched chain acyl-CoA oxidase in humans (113).

In general, xenobiotics with aliphatic chains are excreted as more polar metabolites with a shortened side chain terminating in a carbotyl group. The shortening of the side chain by an even number of carbon atoms indicates that the side chains undergo ω-oxidation followed by β-oxidation. The role of mitochondria and peroxisomes in the degradation of the side chains of xenobiotics has been studied for only a limited number of compounds. In each instance, however, the evidence obtained points to the heavy involvement of peroxisomes (20, 96).

From the above discussion, one can conclude that the main function of mitochondrial β-oxidation is the generation of energy via the degradation of long-chain fatty acids. Peroxisomes, on the other hand, are engaged in the degradation of a wide variety of lipophilic compounds that may have important functions but are not involved in energy provision.

DEGRADATION OF 3-METHYL-BRANCHED FATTY ACIDS: α-OXIDATION

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a multibranched, isoprenoid-derived fatty acid that accumulates in classical Refsum's disease (94) as well as in peroxisome deficiency disorders (24, 117). Phytanic acid is formed from phytol, a multibranched fatty alcohol present in esterified form in chlorophyll. Ruminants ingest large amounts of chlorophyll, and phytol is liberated by the bacteria of the rumen and converted to phytanic acid in the animal's body following absorption (94). Humans probably do not synthesize

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phytanic acid but rather ingest it in their diet (ruminant fat, dairy products). Because a 3-methyl substitution is present, phytanic acid cannot undergo direct β-oxidation. It is first oxidatively decarboxylated in a process called α-oxidation that yields CO_2 and pristanic acid (2), a 2-methyl-branched fatty acid that is β-oxidized in peroxisomes (see above). α-oxidation, which may consist of several consecutive reactions, remains poorly characterized in terms of cofactor requirements and putative intermediates. For instance, the occurrence of an acyl-CoA intermediate (89) or a 2-hydroxy intermediate (101) has been reported by some investigators but denied by others (38, 90). Recent evidence even indicates that formic acid may be liberated in addition to CO_2 (74). In any case, the accumulation of phytanic acid in peroxisome deficiency disorders suggests that peroxisomes may be involved in α-oxidation. However, cell fractionation studies have produced widely conflicting results, perhaps as a consequence of poorly defined assay conditions. Some authors located α-oxidation to the mitochondria (120), whereas others located it to the mitochondria plus the cytosol (66) or to the endoplasmic reticulum (38). In a recent study, Singh et al claimed that α-oxidation is mitochondrial in rodents but peroxisomal in humans (89). Subcellular localization of α-oxidation will likely remain controversial until the reactions are better characterized. Other 3-methyl-branched compounds that are possible substrates for α-oxidation followed by peroxisomal β-oxidation are retinoic acid and dolichols (see below), following their prior conversion to dolichoic acids.

FATTY ACID ELONGATION AND δ4 DESATURATION

Mitochondria and endoplasmic reticulum contain a fatty acid elongation system dependent on acetyl-CoA and malonyl-CoA, respectively. An acetyl-CoA-dependent elongation system has also been described in rat liver peroxisomes (35), but its function remains unknown.

Tissues from patients with peroxisome deficiency disorders exhibit a dramatic decrease in some fatty acids (e.g. 4,7,10,13,16,19-docosahexaenoic acid) considered to be δ4-desaturation products. It is now clear that the δ4-unsaturated bond is not introduced by a δ4-desaturase. Instead, 7,10,13,16,19-docosapentaenoic acid is elongated, possibly in the endoplasmic reticulum, to 9,12,15,18,21 tetracosapentaenoic acid. This process is followed by desaturation at position six to 6,9,12,15,18,21 tetracosahexaenoic acid and by retroconversion by peroxisomal β-oxidation to 4,7,10,13,16,19-docosahexaenoic acid (115).

ETHER GLYCEROLIPID SYNTHESIS

Glycerolipid synthesis begins with the acylation of either glycerol-3-phosphate or dihydroxyacetone-phosphate (53, 99). Acylation of glycerol-3-phosphate,

catalyzed by glycerophosphate acyltransferase, takes place in mitochondria and endoplasmic reticulum. Further conversions that ultimately lead to the synthesis of phospholipids and triacylglycerols are catalyzed by enzymes present in the endoplasmic reticulum. The glycerol-3-phosphate pathway is responsible for the major portion of glycerolipid synthesis and produces glycerolipids in which the fatty acids are linked to the glycerol backbone via ester bonds.

Acylation of dihydroxyacetone-phosphate is catalyzed by dihydroxyacetone-phosphate acyltransferase. This enzyme is present in the peroxisomal membrane and was recently purified from guinea pig peroxisomes (121). Some dihydroxyacetone-phosphate acyltransferase activity also occurs in the endoplasmic reticulum, but it is probably a side activity of glycerophosphate acyltransferase. Acyldihydroxyacetone-phosphate can be reduced by acyldihydroxyacetone-phosphate reductase—an NADPH-dependent enzyme—to 1-acylglycerol phosphate, which is further converted to phospholipids and triacylglycerols by the same enzymes in the endoplasmic reticulum that are mentioned above for the glycerol-3-phosphate pathway. Acyldihydroxyacetone-phosphate reductase has a bimodal subcellular distribution: It is present in the membranes of peroxisomes and endoplasmic reticulum (21). The contribution of the dihydroxyacetone-phosphate pathway to overall ester glycerolipid synthesis appears minor. However, acyldihydroxyacetone-phosphate can be converted via an alternative route, resulting in the synthesis of ether lipids. In this pathway, the fatty acid present in acyldihydroxyacetone-phosphate is replaced by a fatty alcohol, thereby forming an ether bond in a process that gives rise to alkyldihydroxyacetone-phosphate. The reaction is catalyzed by alkyldihydroxyacetone-phosphate synthetase, an enzyme present in the peroxisomal membrane and to a lesser extent in some tissues (or species) in the endoplasmic reticulum (27). Alkyldihydroxyacetone-phosphate is subsequently reduced to 1-alkylglycerol phosphate by acyldihydroxyacetone-phosphate reductase. This enzyme (described above) is therefore often called acyl/alkyldihydroxyacetone-phosphate reductase. 1-alkylglycerol-phosphate is converted to phospholipids that have an ether bond in position-1 or to 1-alkyl-2,3-diacylglycerols in the endoplasmic reticulum by the same enzymes that catalyze the formation of the ester glycerolipids. Acyl-CoA reductase, which reduces acyl-CoA esters to fatty alcohols required for synthesis of the ether bond, is also a peroxisomal membrane protein (6). The peroxisomal enzymes involved in ether lipid synthesis are only slightly or not at all induced by treating rodents with peroxisome proliferators. The importance of peroxisomes in ether lipid synthesis is underlined by the severe lack of ether lipids in tissues from patients with peroxisome deficiency disorders (31).

With the exception of their role as precursors of platelet-activating factor (PAF) and plasmalogens, the function of ether lipids remains largely unknown. Plasmalogens are present in substantial amounts in excitable tissues such as

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heart and brain. It has been estimated that 18% of all human phospholipids consist of plasmalogens (107).

CHOLESTEROL AND DOLICHOL SYNTHESIS

Dolichols are polyisoprenoids, which in animal cells consist of 15 to 23 isoprene units (75 to 115 carbon atoms) (16). Dolichyl phosphate participates in protein glycosylation, but the function of the unphosphorylated dolichols remains unknown. The presence of dolichols in cellular membranes increases membrane fluidity and may facilitate intracellular vesicle fusion and trafficking (16).

The early steps in the biosynthesis of cholesterol and dolichols leading to the formation of farnesyl pyrophosphate are shared by both pathways. The enzymes are cytosolic with the exception of 3-hydroxy-3-methylglutaryl-CoA reductase, the regulatory enzyme of sterol synthesis, which is present in the endoplasmic reticulum. However, some of the enzymes involved in this initial step are also found in peroxisomes. For example, peroxisomes can condense acetyl-CoA to acetoacetyl-CoA, the first reaction in cholesterol and dolichol synthesis (catalyzed in peroxisomes by the 3-ketoacyl-CoA thiolase) (36, 98). They contain 3-hydroxy-3-methylglutaryl-CoA reductase (45) and mevalonate kinase (92). The three enzymes are soluble matrix proteins. Farnesyl pyrophosphate constitutes a branch point. It can be used for protein isoprenylation and serves as a precursor of cholesterol, dolichols, and the side chain of ubiquinone.

The synthesis of cholesterol begins with the condensation of two farnesyl pyrophosphate units catalyzed by squalene synthetase. The conversion of squalene to cholesterol takes place both in peroxisomes and in the endoplasmic reticulum. The synthesis of dolichols is catalyzed by *cis*-prenyltransferase, which adds successive isopentenyl units to farnesyl pyrophosphate. *Cis*-prenyltransferase is present in the endoplasmic reticulum as well as in peroxisomes (15). The peroxisomal enzymes involved in the conversion of farnesyl pyrophosphate to cholesterol and dolichols are membrane bound. Interestingly, peroxisomes also contain the highest intracellular concentration of sterol carrier protein-2 (103), a protein that appears to stimulate the terminal reactions of cholesterol and dolichol synthesis (16). Whether peroxisomal cholesterol and dolichol synthesis serve a specific purpose remains unknown.

PEROXISOME PROLIFERATORS

Structural Diversity

Several structurally diverse chemicals of pharmaceutical, industrial, and agricultural importance induce peroxisome proliferation in hepatocytes of rodents

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and nonrodent species, including primates. Nevertheless, the response in human and nonhuman primates is much less pronounced than that observed in the rat and mouse (57, 75, 79, 80). These agents, designated peroxisome proliferators, include a broad group of chemicals such as hypolipidemic drugs, phthalate ester plasticizers, solvents (e.g. trichloroethylene), herbicides, some leukotriene D_4 antagonists, and the adrenal steroid dehydroepiandrosterone (57, 76, 79, 80). The majority of compounds identified to date as peroxisome proliferators belong to the family of amphipathic carboxylates (e.g. clofibrate) and its analogs, some of which are used in humans as hypolipidemic agents (57, 79, 80). Several of the structural analogs of clofibrate are extremely potent inducers of peroxisome proliferation and hepatocarcinogenesis in rats and mice (77, 80). These include ciprofibrate, methyl clofenapate, nafenopin, gemfibrozil, and fenofibrate, all of which are several orders of magnitude more potent than the prototype compound clofibrate in inducing hypolipidemia, peroxisome proliferation, and liver carcinogenesis (75, 79). Hypolipidemic agents not structurally related to clofibrate are also identified as potent peroxisome proliferators (79). Of this subclass, [4-chloro-6(2,3-xylidino)2-pyrimidinylthio]acetic acid (Wy-14643), 4-chloro-6(2,3-xylidino)2-pyrimidinyl-thio(N-β-hydroxyethyl)acetamide (BR-931), and tibric acid are more potent than clofibrate in inducing peroxisome proliferation and liver tumors in rats and mice (77).

The industrial plasticizers di(2-ethylhexyl)phthalate (DEHP) and di(2-ethylhexyl)adipate (DEHA) are widely used as plasticizing agents in the manufacture of polyvinyl chloride plastics and have been identified as peroxisome proliferators (79). Recently, several nongenotoxic herbicides developed to replace some of the currently used genotoxic herbicides were found to exert potent peroxisome proliferative effects in rats and mice. Thus hypolipidemic drugs, which are increasingly used in the therapeutic control of hyperlipidemia, as well as plasticizers and herbicides with the potential to contaminate the environment may pose a risk to humans (57, 80). Certain anti-inflammatory agents of possible value in the therapeutic and preventive aspects of asthma and allergies are also emerging as peroxisome proliferators (57). In addition, a number of physiological or dietary factors, such as administration of dehydroepiandrosterone, *all-trans* retinoic acid, and high-fat diet can cause peroxisome proliferation (31, 58, 76). The diverse nature of the chemical structures of compounds known to cause peroxisome proliferation and induce the development of liver tumors in rats and mice despite their inability to directly damage DNA raises important questions about the implications to human health of continued disturbances in lipid metabolism caused by sustained activation of peroxisomal β-oxidation system genes (see below).

Pleiotropic Responses—Tissue Specificity

Peroxisomes are widely distributed in most animal and plant cells. Their numbers and volume density remain fairly constant under various physiological

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and pathological conditions. In the liver parenchymal cells of rats, mice, and most other mammals, peroxisomes are few in number and occupy less than 2% of the cytoplasmic volume (63, 67, 68). However, their number increases dramatically in liver cells of rodents following exposure to peroxisome proliferators. As a group, these agents produce predictably similar pleiotropic responses (75). The immediate pleiotropic response is characterized by hepatomegaly with transient hepatocellular hyperplasia, an increase in the number of peroxisomes in hepatic parenchymal cells, and increases in the activity of certain peroxisomal enzymes, especially those involved in the β-oxidation of fatty acids (29, 51, 57, 67). Within a few days of continued treatment, the peroxisome volume density may reach 18–25% of the hepatocyte cytoplasmic volume (63, 67). Similar magnitude of proliferation of peroxisomes in other cell types in rats and mice has not been observed (67, 68). The changes in the levels of peroxisomal enzymes in liver of rats and mice treated with peroxisome proliferators are variable: catalase and urate oxidase activities are increased two- to threefold, whereas the fatty acid β-oxidation enzymes (palmitoyl-CoA oxidase, the multifunctional protein, thiolase) increase 20- to 30-fold (67, 68, 78). The mRNA levels of the three β-oxidation enzymes in the liver of rats treated with peroxisome proliferators increase coordinately 10- to 30-fold (78). Alterations in the numbers of peroxisomes and increases in β-oxidation enzymes and their mRNAs persist as long as peroxisome proliferators are administered (67, 78). The induction of palmitoyl-CoA oxidase, the rate-limiting enzyme in the peroxisomal β-oxidation of straight-chain fatty acids, results in enhanced hepatocellular levels of hydrogen peroxide, which is implicated in the hepatocarcinogenic process (77, 80). Peroxisomal catalase, which is responsible for the detoxification of hydrogen peroxide, is increased less than two- to threefold (67). This differential induction of hydrogen peroxide–generating and –degrading enzymes in the livers of rats and mice exposed to peroxisome proliferators appears to be responsible for the metabolic perturbations leading to the formation of mutagenic 8-hydroxydeoxyguanosine adducts (80).

Dramatic increases in the activities of certain other peroxisomal enzymes, such as carnitine acetyltransferase and carnitine octanoyltransferase, also occur in the livers following peroxisome proliferator treatment. However, these agents induce only a modest increase in acyl-CoA:dihydroxyacetone phosphate acyltransferase activity (17, 27, 42). Additional studies using differential or subtraction hybridization procedures are essential to further isolate and characterize the other possible inducible genes in the liver by continued exposure to peroxisome proliferators. Such studies will enable us to better understand the role played by these in peroxisome proliferator–induced lipid metabolism and liver carcinogenesis (see below).

The levels of certain extraperoxisomal enzymes, in particular all three members of the microsomal p450 IVA family (IVA1, IVA2, and IVA3), which

exhibit ω-hydroxylase activity (23), are also increased in response to peroxisome proliferators, but the magnitude of increase is between two- and fivefold, unlike the more than 30-fold increase in the activity of the β-oxidation enzymes. As pointed out elsewhere, the p450 IVA family of enzymes metabolizes a variety of substances, including prostaglandins E_1 and $F_{2\alpha}$ and certain fatty acids (23, 106). Soluble epoxide hydrolase (sEH), a homodimeric protein with an estimated native M_r of 120,000 and an apparent subunit M_r of 60,000, is induced in rat liver concomitantly with the peroxisomal β-oxidation by peroxisome proliferators (26). In rat liver, the constitutive expression of sEH is low, but treatment with peroxisome proliferators results in a 5- to 10-fold increase in its mRNA level (26). sEH hydrates a wide range of substrates and is crucial in the degradation of potential toxic disubstituted aliphatic epoxides (26).

From a theoretical perspective, this maximum level of peroxisome proliferation and the induction of the β-oxidation enzyme system, *p450 IVA* and *sEH*, by stimulation of expression of their respective genes, should be achieved in the liver with any xenobiotic classified as a peroxisome proliferator, provided that a maximally effective dose is administered for a period of 10–14 days (63, 67, 68). It is now well established that all peroxisome proliferators tested thus far in long-term studies have been found to induce liver tumors, despite the fact that these agents do not directly cause DNA damage (57, 75, 77, 79, 80, 119). The hepatocarcinogenicity of peroxisome proliferators is therefore considered a delayed pleiotropic response resulting from sustained increases in the hydrogen peroxide–generating peroxisomal β-oxidation system (75). Peroxisome proliferators are not overtly toxic to the liver, and the hepatomegaly induced by these compounds is typically adaptive in nature without inducing cell necrosis and steatosis. None of the currently identified peroxisome proliferators is a classical mutagenic agent (119). Any peroxisome proliferator capable of increasing peroxisome volume density to 18–25% of the cytoplasmic volume level is likely to induce liver tumors in rats and mice on long-term exposure at that dose level.

The demonstration of essentially similar morphological and biochemical changes in liver following exposure to structurally diverse peroxisome proliferators is a strong indication of the existence of a common mechanism that mediates the coordinated induction response (see below). The hypothesis that sustained induction of peroxisome proliferative response is the basis for the delayed toxicity/carcinogenicity raises some pointed questions about the tissue specificity of this immediate pleiotropic response and its presumed implications (67, 68, 75, 80). One such implication is that the carcinogenicity in animals exposed to peroxisome proliferators should manifest only in organs that exhibit this pleiotropic response. The excellent correlation between the inducibility of peroxisome proliferation and carcinogenicity of this class of

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xenobiotics has led investigators to postulate that sustained induction of the peroxisomal fatty acid β-oxidation system and the resultant increases in H_2O_2 and the reactive oxygen intermediates form the basis for carcinogenesis (77, 79, 80).

Detailed assessment of the extent of increase in peroxisomal β-oxidation enzyme activities and of the mRNAs that encode the three enzymes of this system in various rat tissues following treatment with ciprofibrate, a peroxisome proliferator, revealed maximal induction in liver (> 20-fold) but only a two- to fourfold increase in mRNAs in the kidney, small intestine, and heart (68). Ciprofibrate did not significantly increase the levels of these mRNAs in other organs such as lung, brain, testis, adrenal, skeletal muscle, spleen, and pancreas (68). Thus the marked inducibility of the β-oxidation system genes in liver and the subsequent development of liver tumors support the hypothesis that tumors develop in tissues that exhibit maximum inducibility of peroxisome proliferation vis-à-vis fatty acid β-oxidation system following exposure to peroxisome proliferators (63, 68, 80).

Mechanism of Induction of Peroxisomal β-Oxidation Enzymes: Receptor-Mediated Cell-Specific Effects

The differences in the magnitude of induction of β-oxidation enzymes coded for by their respective genes in different tissues must result from cell-specific factors. Cell-specific effects of peroxisome proliferators have been documented very succinctly by studies on induction in vitro in primary liver cell cultures, in hepatocytes transplanted in s.c. fat or in the anterior chamber of the eye of rats, and in rats with transdifferentiated pancreatic hepatocytes (68, 75, 80). Because peroxisome proliferation occurs in hepatic parenchymal cells irrespective of their location in the animal but not in other adjacent or contiguous nonhepatic cells in these extrahepatic locations, hepatic parenchymal cells therefore must have a mechanism that enables them to recognize these xenobiotics and respond to them appropriately (49, 50, 79). Furthermore, the similarity of biological responses to agents with little apparent structural relationship and the rapid and coordinated transcriptional induction of the nuclear genes encoding the three peroxisomal enzymes of the β-oxidation pathway (palmitoyl-CoA oxidase, enoyl-CoA hydratase/3-hydroxyacyl CoA dehydrogenase, and 3-ketoacyl-CoA thiolase) led to the proposition that soluble receptor(s) induce the expression of peroxisomal genes (49, 78, 79). The first indication of such a receptor-mediated mechanism was the demonstration of specific soluble-binding moiety for peroxisome proliferators in liver cytosol (49). In addition, the distribution of [^{14}C]ciprofibrate—found almost exclusively in liver—as observed by whole-body autoradiography is also consistent with the tissue-specific biological responses (116).

In keeping with the proposed receptor-mediated mechanism, a mouse per-

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oxisome proliferator–activated receptor, mPPAR, was recently isolated (39). A high level of expression of mPPAR mRNA was observed in liver, kidney, and heart, but its expression in other tissues was very weak (39). The pattern of expression of PPAR in mouse thus mirrors the tissue-specific effects of peroxisome proliferators (68). mPPAR is a member of the nuclear hormone (orphan) receptor superfamily and is therefore a putative transcription factor (39). Three novel members of the nuclear hormone receptor family from *Xenopus laevis* that are structurally and functionally related to the mPPAR were subsequently cloned (14). These *Xenopus* PPARs are designated xPPARα, xPPARβ, and xPPARγ (14). The cDNAs of xPPARα and xPPARβ were isolated from an oocyte library, whereas the xPPARγ cDNA was isolated from frog liver (14). The pattern of expression of xPPARγ is similar to that of mPPAR, coinciding with tissue responsiveness to peroxisome proliferators in rodents (14). A peroxisome proliferator–activated receptor from rat (rPPAR) and human was recently isolated (21, 25, 84b), and a new member from mouse liver (mPPARγ) that exhibits 75% amino acid similarity to xPPARγ has also been cloned (125). The presence of two PPARs in mouse liver (mPPARα and mPPARγ) suggests the possibility of multiple signaling pathways for peroxisome proliferator–induced pleiotropic responses (125). These PPARs may exhibit differential response/specificity to different peroxisome proliferators, or they may participate in a complex interaction(s) with other nuclear factors in transcriptional activation (see below). The comparison of predicted amino acid sequence of various PPARs isolated to date reveals that the DNA-binding domains and the ligand-binding domains are best conserved between various members of the PPAR family (125).

Peroxisome proliferators have been shown to activate PPARs derived from different species in transient expression systems (14, 39, 123, 125). Physiological agents, in particular fatty acids such as arachidonic acid, lauric acid, and linoleic acid, also activate PPARs (25, 46). Other physiological agents such as dehydroepiandrosterone (DHEA), DHEA sulfate, and cholesterol failed to do so (4, 25, 39). It is of particular interest that the synthetic arachidonic acid analog 5,8,11,14-eicosatetraynoic acid exhibited 50–100 times more potency in activating xPPARα than did the peroxisome proliferator Wy-14643 (25). The failure of DHEA to activate mPPAR (39) and rPPAR (25) is intriguing and raises the possibility of multiple signaling mechanisms for the induction of the fatty acid β-oxidation system.

The identification of PPARs has enabled the initiation of studies to elucidate the mechanisms by which structurally diverse peroxisome proliferators induce the coordinated transcription of the genes that encode the peroxisomal β-oxidation enzymes (78). *Cis*-acting peroxisome proliferator–responsive elements (PPREs) have recently been characterized in the upstream regions of both palmitoyl-CoA oxidase and the multifunctional enoyl-CoA hydratase/3-hy-

droxyacyl-CoA dehydrogenase genes of rat (104, 123). A PPRE element has also been recognized in the rat thiolase gene (47). The gene for cytochrome P450IVA6 (CYP4A6), which catalyzes ω-1 hydroxylation of fatty acids, also contains a PPRE in its 5′-flanking sequence (65). Available evidence reveals that diverse PPARs recognize the PPRE motif TGACCT (or a variant thereof), located upstream of peroxisome proliferator–responsive genes (47, 123, 124).

The role of PPARs in transcriptional activation of peroxisome proliferator–responsive genes and the interplay between PPARs and other transcription factors require elucidation in light of the failure of the ligand to bind the PPARs (39). Evidence for a convergence of the peroxisome proliferator and retinoid

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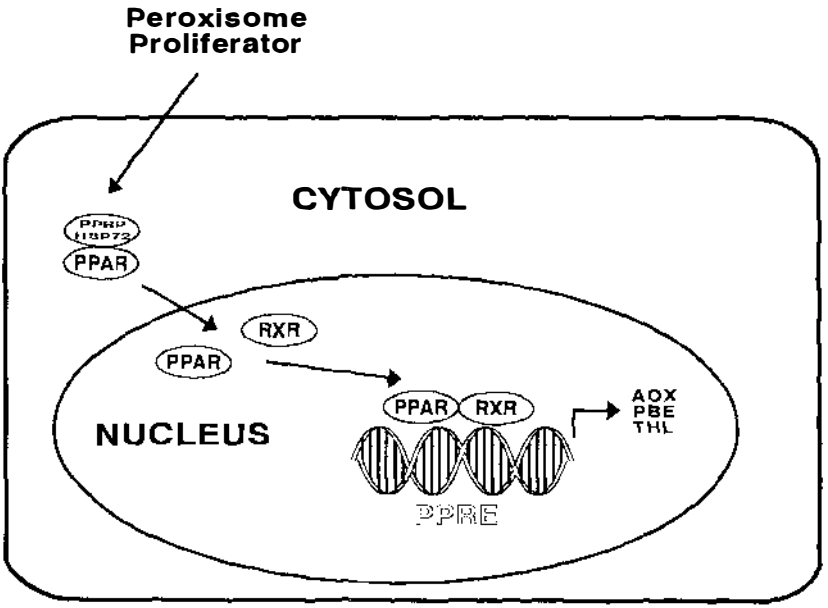

Figure 1 Model of receptor-mediated induction of peroxisome proliferator pleiotropic responses. This model depicts the mechanism for activation of β-oxidation genes by the peroxisome proliferator–activated receptor (PPAR) as well as its interaction with peroxisome proliferator–binding proteins (PPBP/HSP72) and with the 9-*cis* retinoic acid receptor (RXR). The peroxisome proliferator–response element (PPRE) contains directly repeated TGACCT-like motifs. TGACCT repeat separated by a single nucleotide is found in all three genes for the β-oxidation system: palmitoyl-CoA oxidase (AOX), the peroxisomal multifunctional enzyme enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (PBE), and thiolase (THL). These PPREs all conform to RXR elements. mPPARa and rPPAR heterodimerize with RXRα on PPRE to synergistically activate transcription. This figure depicts PPBP/HSP72 complexing with PPAR in the cytosol. Peroxisome proliferators diffuse into the cytosol to bind this complex and translocate to the nucleus, where peroxisome proliferators facilitate dimerization of PPAR with RXRα for transcriptional activation. Alternately, only PPBP/HSP72 complex—not PPAR—exists in the cytosol. This complex is responsible for translocating the peroxisome proliferator to the nucleus, where it activates PPAR.

signaling pathways was recently obtained (21, 47). mPPAR and rPPAR have been shown to form heterodimeric complex with 9-*cis* retinoic acid receptor, RXRα (21, 47). The PPAR-RXRα complex has been shown to activate gene expression in a peroxisome proliferator–dependent manner by binding to the direct repeat in the PPRE of the palmitoyl-CoA oxidase gene. Because neither PPAR nor RXRα exhibits peroxisome proliferator–binding ability, other peroxisome proliferator–binding proteins (PPBPs) (1, 50) may be involved in peroxisome proliferator–mediated signaling (Figure 1). PPBPs were isolated using clofibric acid, nafenopin, or ciprofibrate affinity chromatography (1, 50). One of the PPBPs was identified as HSP72, and evidence now suggests that HSP72 also interacts with rPPAR (37). Baculovirus-insect cell–expressed PPAR binds to a clofibric acid affinity matrix along with the HSP72 (37). HSP 72 and rPPAR elute simultaneously from the clofibrate-sepharose matrix in a clofibric acid–dependent manner (37). HSP72-PPAR complex may be involved in translocating the ligand to the nucleus, where the peroxisome proliferator may activate PPAR/RXRα complex or assist in heterodimerization for interacting with the PPRE (Figure 1). The convergence of a variety of factors may eventually dictate the tissue specificity of peroxisome proliferator–induced pleiotropic responses.

DISORDERS OF PEROXISOMAL LIPID METABOLISM

At present, 15 different peroxisomal diseases have been identified. Excluding acatalasemia, hyperuricemic gout, and hyperoxaluria type I, all of these disorders exhibit disturbances in lipid metabolism. Disorders of peroxisomal lipid metabolism are often characterized by multiple anatomical and functional deficits, including severe neurological abnormalities (24, 117).

Peroxisomal disorders can be subdivided into three categories (117). The first group, the peroxisome deficiency disorders [Zellweger syndrome, neonatal adrenoleukodystrophy (ALD), infantile Refsum's disease, and hyperpipecolic acidemia], lacks morphologically recognizable peroxisomes (or has a greatly reduced number of normal peroxisomes) and displays an almost generalized loss of peroxisomal functions. These diseases are apparently caused by a defect in peroxisome biogenesis. Peroxisomal proteins are synthesized on free polyribosomes in the cytosol and are posttranslationally imported in preexisting peroxisomes, which are thought to multiply by fission or budding (53). Most peroxisomal proteins are synthesized at their mature size. Several of the matrix proteins carry a peroxisomal targeting signal consisting of three amino acids (Ser-Lys-Leu or conserved variants) at their extreme carboxy terminus (95). However, the targeting signal of most membrane proteins and of a number of matrix proteins (e.g. catalase) remains unknown. Peroxisomal thiolase is synthesized as a precursor possessing a cleavable

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amino terminal presequence that functions as a peroxisomal targeting signal for this protein (95).

How peroxisomal proteins are imported and which membrane polypeptides constitute the protein translocation machinery is not well understood. Complementation analysis following somatic cell fusion of fibroblasts from patients with peroxisome deficiency disorders indicates that at least nine gene products are involved in peroxisome biogenesis (86). In peroxisome deficiency disorders, peroxisomal proteins are synthesized at their normal rate. They are not found in the particulate fraction of the cell but in the cytosol, which indicates that they were not imported into peroxisomes (82, 95). Some unimported proteins (e.g. the β-oxidation enzymes) appear to be rapidly degraded in the cytosol. Interestingly, seemingly empty vesicular structures that contain some but not all of the peroxisomal integral membrane proteins and that may also contain the unprocessed thiolase precursor are found in the tissues of some Zellweger patients (19, 82). The presence of these "peroxisomal ghosts" suggests that more than one import route may exist and that not necessarily all routes are defective in peroxisome deficiency disorders. In keeping with the metabolic function of peroxisomes described earlier in this article, the degradation of very long-chain fatty acids, phytanic and pristanic acids, and eicosanoids is impaired in patients with peroxisome deficiency disorders. The synthesis of bile acids and ether lipids is also impaired. Consequently, these patients accumulate very long-chain fatty acids as well as pristanic and phytanic acids and do not excrete shortened eicosanoid metabolites in urine. Rather, they excrete the bile acid intermediates di- and trihydroxycoprostanic acids in bile, and their tissues display a severe lack of ether lipids (24, 117). How these defects in lipid metabolism relate to the multiple anatomical and functional deficits remains unknown.

A second group of peroxisomal disorders is characterized by a loss of multiple peroxisomal functions, but normal peroxisomes are present. An example is rhizomelic chondrodysplasia punctata, in which ether lipid synthesis and phytanic acid oxidation are disturbed (24, 117).

In the last group of peroxisomal diseases, a single peroxisomal enzyme is deficient. X-linked ALD, the most frequent peroxisomal disease, affects one out of 20,000 males and is a member of this group. In X-linked ALD, the degradation of very long-chain fatty acids is disturbed, probably owing to a deficiency of the peroxisomal very long-chain acyl-CoA synthetase (55). Recently, the gene responsible for the disease was cloned (64). The deduced amino acid sequence of the gene product exhibits significant homology with a previously characterized 70 kDa ATP-binding peroxisomal integral membrane protein. The relationship between the X-linked ALD gene product and the deficient peroxisomal very long-chain acyl-CoA synthetase activity has not been determined. Other examples of single peroxisomal enzyme deficien-

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cies are palmitoyl-CoA oxidase deficiency, in which the degradation of very long-chain fatty acids is impaired, and deficiencies of the multifunctional protein and the 3-ketoacyl-CoA thiolase, in which not only the degradation of very long-chain fatty acids is impaired, but also that of pristanic acid as well as the synthesis of the bile acids. Accumulation of pristanic acid and of the bile acid intermediates di- and trihydroxycoprostanic acids in the presence of normal levels of very long-chain fatty acids in some patients suggests a branched-chain acyl-CoA oxidase deficiency (113). Whether classical Refsum's disease, in which the α-oxidation of phytanic acid is impaired, can be classified as a peroxisomal disease will remain speculative until the exact subcellular localization of α-oxidation is established.

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SUMMARY

Peroxisomes are present in virtually all eukaryotic cells. At present, they are known to contain more than 50 enzymes, more than half of which play a role in lipid metabolism. During the past two decades, considerable knowledge has been gained about the role of peroxisomes in lipid metabolism, the implications of induction of hepatic peroxisome proliferation and the peroxisomal fatty acid β-oxidation enzyme system in the development of hepatocellular carcinomas in rats and mice by structurally diverse groups of chemicals designated as peroxisome proliferators, and the biochemical basis for inheritable diseases in humans caused by disturbances and/or deficiencies in peroxisomal lipid metabolism. Nevertheless, many unanswered questions remain. The ontogeny and homeostatic interrelationships between the enzymes of the peroxisomal and mitochondrial lipid metabolism have yet to be fully elucidated.

The mechanism(s) by which PPARs are activated also remains unclear. Information about the interplay between PPAR, RXRα, HSP72, and other possible regulatory molecules is necessary to elucidate the transcriptional activation of inducible peroxisomal genes. The assumption that multiple signaling pathways may be responsible for the pleiotropic responses induced by structurally different peroxisome proliferators requires further examination. Finally, studies to identify and characterize different PPARs and PPREs from inducible peroxisomal genes from a variety of species are also required for a clear understanding of the role of peroxisomal β-oxidation enzyme system in the pathogenesis of hepatocellular carcinogenesis induced by peroxisome proliferators.

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