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Peroxisomes in Lipid Metabolism

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Abstract Gene targeting and the elucidation of mutations underlying inherited peroxisomal diseases have provided new insights in peroxisomal lipid metabolism in vivo. The work led to the identification of a novel peroxisomal β-oxidation pathway and established clearly that genes, which are required for efficient peroxisomal oxidation of fatty acids, at the same time are key regulators of PPARα function in vivo. The new mouse models may provide helpful tools in the search for unknown natural PPARα agonists and in screening for in vivo PPARα antagonists. J. Cell Biochem. Suppls. 30/31:158–167, 1998. © 1998 Wiley-Liss, Inc.

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More than one-half of all known peroxisomal enzymes have a role in lipid metabolism. They catalyze the oxidation of fatty acids and related substrates, the detoxification of glyoxylate and various xenobiotics, and several reactions in the biosynthesis of plasmalogens, bile acids, and docosahexaenoic acid. Several enzymes involved in cholesterol biosynthesis have been identified within this organelle [Krisans, 1996]. Peroxisomes are also the principal site of H_2O_2 metabolism, catalyzed by β- and ω-oxidases and catalase. One major function of peroxisomes is to protect cells against oxidative stress by the compartmentalization of most H_2O_2 metabolism within the organelle. The H_2O_2 thus produced is efficiently decomposed within the organelle by catalase. Any H_2O_2 escaping from peroxisomes could encounter secondary protective systems in the cytosol. A second function of peroxisomal respiration is the disposal of excess reducing equivalents. The peroxisomal oxidases are free of the constraint imposed by respiratory control, which regulates mitochondrial activity. Therefore, they may function even when the intracellular ATP : ADP ratio is high [for review, see De Duve, 1969; De Duve and Baudhuin, 1966]. A third potential function of peroxisomal respiration may be to contribute to thermogenesis and regulation of the energy balance. Since the energy of the peroxisomal oxidations is not conserved as ATP, a large fraction of this energy is dissipated as heat. Peroxisomes proliferate strikingly in brown adipose tissue during cold adaptation [Ahlabo and Barnard, 1971] despite the fact that the principal means of heat production in this specialized tissue is attributed to the burning of fatty acids in uncoupled mitochondrial β-oxidation [Smith and Horwitz, 1969].

The specificity of peroxisomal β-oxidation overlaps that of mitochondrial β-oxidation. However, there are substrates that are preferentially or exclusively oxidized in peroxisomes, such as very-long-chain fatty acids (VLCFA), polyunsaturated fatty acids, methyl-branched fatty acids, dicarboxylic fatty acids, prostaglandins, and the cholesterol side chain in bile acid synthesis. Although the chemical modifications made to the fatty acyl-coenzyme A (CoA) during peroxisomal and mitochondrial β-oxidation are identical, the enzyme proteins involved are all nonidentical. Peroxisomal β-oxidation starts with introduction of a Δ2,3-double bond catalyzed by acyl-CoA oxidase (ACO), which consumes O_2 and leads to the production of H_2O_2. Next, the consecutive addition of H_2O and NAD-

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dependent oxidation is catalyzed by Δ2,3-enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, which combines both enzymatic activities in a single polypeptide (peroxisomal bifunctional enzyme [PBE]). The resulting product, 3-ketoacyl-CoA, is cleaved thiolytically through the action of a peroxisomal 3-ketoacyl-CoA thiolase, yielding one molecule of acetyl-CoA and the (n-2) lower homologue acyl-CoA, which can enter a new round of β-oxidation. Peroxisomal β-oxidation of straight-chain fatty acids does not go to completion but stops at the level of octanoyl-CoA, which is exported from the peroxisome and oxidized to completion in mitochondria [for review, see Lazarow and Moser, 1995].

Foerster et al. [1981] and Handler and Thurman [1988] showed that the conventional mitochondrial substrates, e.g., palmitate (C16:0) or oleate (C18:1), are used almost exclusively by mitochondrial β-oxidation under most physiologic circumstances. VLCFAs such as erucic acid (C22:1), are much more likely to be subjected to peroxisomal chain-shortening because the mitochondrial β-oxidative capacity is low for these substrates and their acyl-CoA esters would therefore readily accumulate in the cytosol. This is in line with metabolic abnormalities observed in patients with Zellweger disease or adrenoleukodystrophy in whom accumulation of VLCFAs often parallels striking hypolipidemia with respect to the conventional long-chain fatty acids, presumably as a result of compensatory induction of mitochondrial β-oxidation [Lazarow and Moser, 1995].

FUNCTIONAL DIVERSITY OF PEROXISOMAL β-OXIDATION PATHWAYS

An important question was whether the above described β-oxidation pathway would be sufficient for oxidation of all substrates that are oxidized in peroxisomes in vivo. Compared with the mitochondrial pathway, the substrate specificity of peroxisomal β-oxidation is exceedingly broad. On the basis of this consideration, it was not surprising that several new cDNAs, encoding proteins that were related to the conventional peroxisomal β-oxidation enzymes, were cloned during the past few years. These include 3α,7α,12α-trihydroxycholestanoyl-CoA oxidase [Pedersen et al., 1997], pristanoyl-CoA oxidase [Vanhooren et al., 1996], the so-called 17β-hydroxysteroiddehydrogenase (type IV) [Leenders et al., 1994a,b], and sterol carrier protein-x (SCPx) [Seedorf and Assmann, 1991]. The latter two appeared particularly interesting, because they exhibited an unusually complex genetic organization and a surprising multifunctionality that was absent from most other known peroxisomal activities.

SCPx was discovered upon cloning and sequencing of sterol carrier protein-2 (SCP2) cDNAs [Seedorf and Assmann, 1991]. The name "sterol carrier protein" relates to the fact that SCP2 was isolated originally as a "cytosolic" factor required for efficient in vitro conversion of 7-dehydrocholesterol to cholesterol, catalyzed by membrane-bound sterol-Δ7-reductase in the ER [Noland et al., 1980]. The protein is identical to the nonspecific lipid transfer protein (ns-LTP), which was purified as phospholipid transfer protein, catalyzing the exchange of a variety of phospholipids between membranes in vitro [Blaj and Zilversmit, 1977]. More recent studies employing antisense oligonucleotides and overexpression of SCP2 cDNA in cell lines suggested participation of SCP2 in cytosolic cholesterol transport to the plasma membrane in these cells [Puglielli et al., 1995; Baum et al., 1997]. On the basis of these and other in vitro studies, it was hypothesized that SCP2 functions as potentially important mediator of cholesterol transport through the cytosol to various locations in the cell, such as the plasma membrane, mitochondria, and the endoplasmic reticulum (ER) [reviewed in Pfeifer et al., 1993]. However, low expected activity under physiological conditions, apparent lack of specificity for cholesterol, and the predominant localization of SCP2 in peroxisomes made it difficult to understand how the protein might carry out its proposed role in cytosolic free cholesterol trafficking in the intact cell.

RELATIONSHIP OF SCP2/SCPX AND THE 17β-HYDROXYSTEROID DEHYDROGENASE IV WITH THE PEROXISOMAL β-OXIDATION SYSTEM

The SCP2-encoding gene (Scp2) comprises 16 exons, which span ~100 kb on human chromosome 1p32 [Yamamoto et al., 1991; Raabe et al., 1996; Ohba et al., 1994]. Transcription initiation is controlled by two distant promoters that were mapped immediately upstream of the first exon (P1) and exon 12 (P2) [Ohba et al., 1995]. P2 is used to generate SCP2 encoding transcripts, which combine the coding information provided by exons 12–16. In addition, alternate transcription initiation at P1 leads to produc-

tion of a second transcript that includes the coding information provided by exons 1–16. The respective gene product consists of 547 amino acids and was named sterol carrier protein-x (SCPx). SCPx represents a fusion protein between a thiolase domain, extending from amino acids 1–404, and SCP2, which is located at the C-terminus [Ossendorp et al., 1990]. The fused SCPx-encoding gene is present in all vertebrates and could be traced back to *Drosophila melanogaster* [Pfeifer et al., 1993] (Genbank Accession No. X97685). By contrast, two separated genes for SCP2 and the thiolase were identified in *Caenorhabditis elegans* and several yeast species [Bunya et al., 1997; Tan et al., 1990]. Interestingly, an ancient precursor of SCP2 could be identified even in the primitive methanogenic archaeon, *Methanococcus jannaschii* [Bult et al., 1996].

It is known from in vitro studies that SCPx has similar lipid transfer activity as SCP2 and that the substrate specificity of the SCPx thiolase shows a preference for straight medium-chain acyl-CoA substrates and tetramethyl-branched 3-ketopristanoyl-CoA [Seedorf et al., 1994; Wanders et al., 1997]. Thus, the properties of the SCPx-associated thiolase differ from the initially identified peroxisomal thiolase that is assumed traditionally to play a prominent role in peroxisomal β-oxidation of most natural substrates, including bile acids and VLCFA [Schram et al., 1987; Hijikata et al., 1987].

The structure of SCPx is similar to that of another peroxisomal protein in which a C-terminal SCP2-like domain is fused to a domain leading to acyl-CoA 2-enoyl hydratase/3-hydroxyacyl-CoA dehydrogenase (peroxisomal bifunctional protein, PBE) activity. The fused peptide is part of the 80-kDa precursor of 17β-hydroxysteroid dehydrogenase type IV (17β-HSD4), a multifunctional, multidomain protein consisting of an N-terminal SCAD domain that harbors 17β-HSD and 3-OH-acyl-CoA dehydrogenase activity, followed by the acyl-CoA 2-enoyl-hydratase domain and the C-terminal SCP2-like domain [Leenders et al., 1996]. Although all the domains are functionally active in the fused protein [Leenders et al., 1996; Seedorf et al., 1995], processing occurs after import into peroxisomes at the junction between the SCAD and acyl-CoA 2-enoyl-hydratase domains [Markus et al., 1996]. The SCP2-like domain is required for import of the 80-kDa precursor into peroxisomes and confers a similar intrinsic lipid transfer activity to the PBE-SCP2 fusion protein as was demonstrated for SCPx [Leenders et al., 1996]. However unlike SCPx, which is partly cleaved post-translationally at the thiolase-SCP2 junction [Seedorf et al., 1994], the SCP2-like domain is not separated from the acyl-CoA 2-enoyl-hydratase domain in 17β-HSD4. Also mechanisms leading to separate expression of the SCP2-like peptide (i.e., alternative splicing pathways or alternative transcription initiation) could not be demonstrated. Despite the differences that exist between the two genes, both are reminiscent of bacterial operons, in which distinct functions are combined in a common transcriptional unit. One evident consequence of their structures is that the SCP2 domains are strictly co-expressed with the enzymatic activities. Coordinate expression of an SCP2 homologue with the peroxisomal β-oxidation system was also observed in *Candida tropicalis*, in which two separated genes encode the SCP2 homologue (PXP18) and the thiolase. In this yeast, PXP18 is co-induced with the peroxisomal β-oxidation system after addition of oleic acid to the culture medium [Tan et al., 1990]. It appears noteworthy that purified SCP2 bound most fatty acids and fatty acyl-CoAs with similar or even higher affinity than it did sterols in a recently published study [Stolowich et al., 1997].

In contrast to the well known PBE enzyme (also called L-bifunctional protein) [Osumi et al., 1980], which converts *trans*-enoyl-CoA to their 3-keto derivatives via the L-hydroxy stereoisomer, the new PBE enzyme catalyzes the same transformation via the D-stereoisomer [Jiang et al., 1996, 1997]. The two PBE proteins differ also in their substrate specificity. Whereas the D-PBE catalyzes the formation of 3-ketoacyl-CoA intermediates from straight and 2-methyl-branched fatty acyl-CoAs, the activity of L-PBE is only high with the straight-chain substrates [Jiang et al., 1996, 1997; Dieuaide-Noubhani et al., 1996; Qin et al., 1997]. Recently, van Grunsven et al. [1998] identified a patient with isolated D-PBE deficiency. In addition to a severe block in pristanic acid β-oxidation (methyl-branched fatty acid), the biochemical abnormalities of the patient indicated that the enzyme is required for efficient peroxisomal degradation of the cholesterol side chain in bile acid synthesis. A surprising finding was that D-PBE deficiency also affected peroxisomal β-oxidation of VLCFA-CoA. Although secondary causes can-

not be excluded, this finding suggests that D-PBE, together with L-PBE, may play an important role also in β-oxidation of VLCFA-CoA substrates.

ABNORMAL PEROXISOMAL METABOLISM OF METHYL-BRANCHED FATTY ACYL-COA IN SCP2 KNOCKOUT MICE

Extensive efforts in several laboratories to identify human inherited diseases that would result from *Scp2* mutations were not successful. Therefore, the biological function of *Scp2* was investigated by employing gene targeting in mice [Seedorf et al., 1998]. The knockout (KO) mouse model demonstrated normal adrenal morphology, essentially normal plasma lipids, absence of developmental abnormalities or salt wasting, and no abnormalities affecting fertility, which differed very clearly from all known human inherited diseases affecting steroidogenesis, cholesterol synthesis, or cholesterol trafficking. On the other hand, cholesterol ester storage pools were markedly depleted in the liver of knockout mice. But it was shown that the effect was not specific for cholesterol because free fatty acid and triglyceride concentrations were also decreased proportionally. Thus, depletion of cholesterol esters seemed to relate to decreased availability of free fatty acids required for intracellular esterification, rather than a specific abnormality in cytosolic free cholesterol trafficking.

Gas chromatographic fatty acid analyses with saponified lipid extracts from plasma and liver from KO mice showed no significant differences regarding the relative levels of the straight long-chain saturated, monounsaturated, polyunsaturated or VLCFA. However, 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid) was significantly elevated in (−/−) mice compared with controls. Phytanic acid is a terpene fatty acid which is produced in heterotrophic organisms from plant-derived phytol (an isoprenoic alcohol esterified to ring IV of chlorophyll; cf. Fig. 1). Since neither phytanic acid nor phytol is synthesized de novo in mammals, phytanic acid serum concentrations depend on dietary intake of performed phytanic acid or its precursor phytol, storage of phytanic acid in cellular neutral lipids and the catabolic rate of phytanic acid [Steinberg, 1995]. Excessive storage of phytanic acid is known to occur also in Refsum disease, an autosomal recessive disorder associated with severe neurologic impairment in affected patients. The disease was shown recently to be caused by mutations in the gene encoding phytanoyl-CoA α-hydroxylase (PHYH) [Jansen et al., 1997; Mihalik et al., 1997].

When SCP2 (−/−) mice were exposed to semisynthetic diets supplemented with phytol (the metabolic precursor of phytanic acid), phytanic acid levels increased much more pronouncedly (≥1 mmol/L of plasma) in Scp2 (−/−) mice than in controls. In parallel, the transgenic strain developed severe anomalies, such as a negative energy balance (drastic weight loss with normal food intake), neurologic abnormalities, hypolipidemia, hypoglycemia, and premature death, not seen in controls [Seedorf et al., 1998].

The normal catabolism of phytol is illustrated schematically in Figure 1. It begins with conversion to phytanic acid, followed by activation to phytanoyl-CoA in the cytoplasm. Phytanoyl-CoA is then imported into peroxisomes, followed by α-oxidation, which involves hydroxylation at the α-carbon position by phytanoyl-CoA hydroxylase (PHYH). Subsequently, 2-OH-phytanoyl-CoA is converted to pristanic acid [Croes et al., 1997]. Pristanic acid is activated to pristanoyl-CoA, which is then subject to six cycles of peroxisomal β-oxidation. The intermediates of the first cycle are Δ2,3-pristenoyl-CoA (produced by pristanoyl-CoA oxidase), 3-OH-pristanoyl-CoA, and 3-ketopristanoyl-CoA (produced by a peroxisomal bifunctional enzyme). Finally, 3-ketopristanoyl-CoA is substrate for thiolytic cleavage, catalyzed by a 3-ketopristanoyl-CoA thiolase, which yields the (n-3) lower homologue of pristanoyl-CoA (4,8,12-trimethyltride-canoyl-CoA) and propionyl-CoA [reviewed by Steinberg, 1995]. Although studies on mice are not available, it now appears that this pathway operates similarly in rats and humans [Watkins et al., 1994; Singh and Poulos, 1995].

Abnormal activation of phytanic acid was unlikely to explain the drastic increase of phytanic acid in Scp2 (−/−) mice because recent studies demonstrated convincingly that phytanoyl-CoA ligation is mediated by a common long-chain fatty acyl-CoA ligase [Watkins et al., 1996], whereas metabolism of long straight-chain fatty acids was not inhibited by the gene disruption. PHYH was expressed normally in Scp2 (−/−) mice, thus excluding secondary down-regulation of PHYH expression. As indicated schematically in Figure 1, the

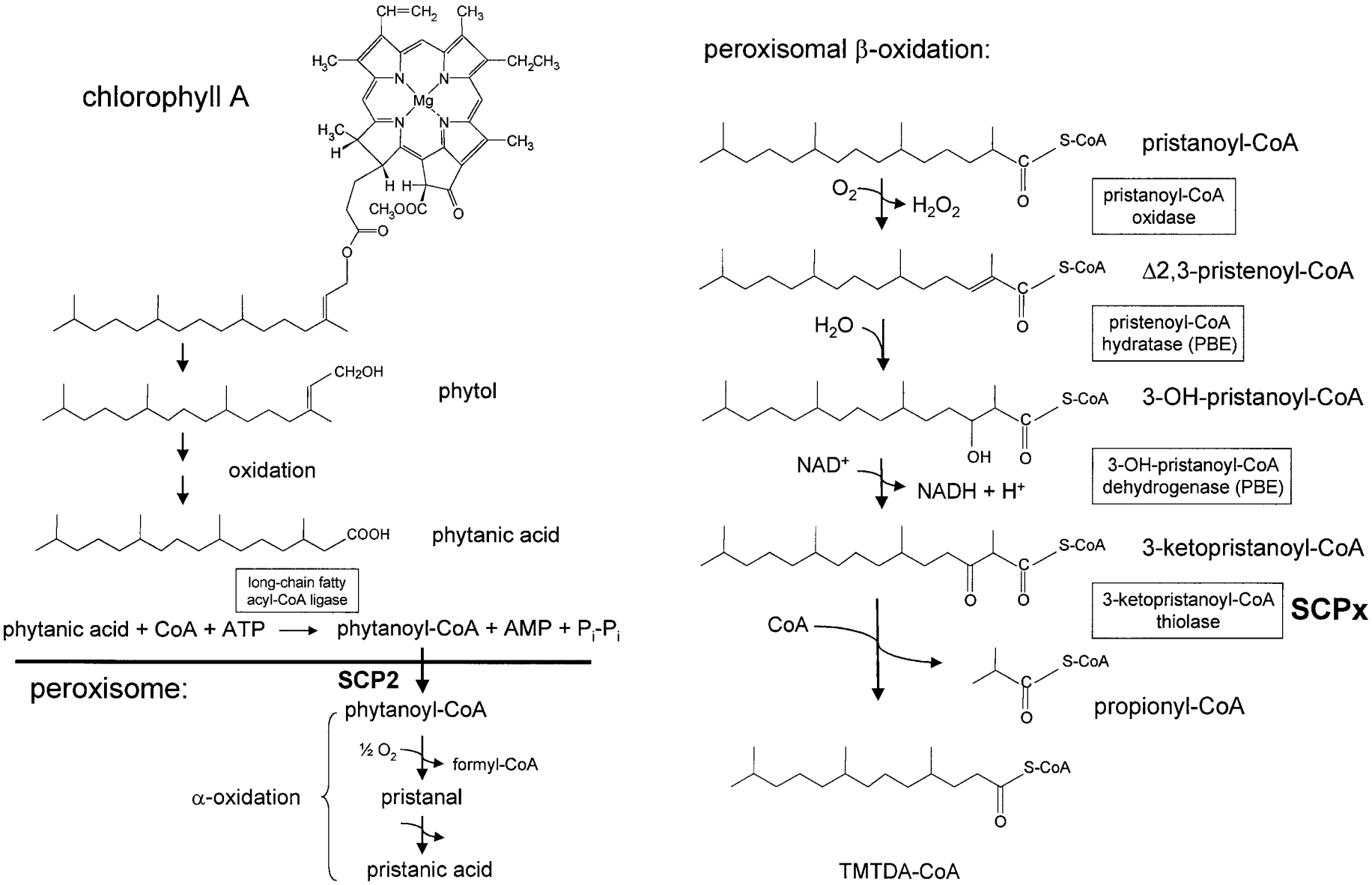

Fig. 1. The roles of SCP2 and the SCPx thiolase in mammalian phytol metabolism. The PPARα agonist phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) results either directly from the diet or from oxidation of dietary phytol. Note that phytanic acid must be decarboxylated to pristanic acid before entering peroxisomal β-oxidation, because the β-carbon atom is blocked by the 3-methyl group. Presumed intermediates of α-oxidation are 2-OH-phytanoyl-CoA and pristanal, but the precise cofactor requirements are unknown. Enoyl-CoA hydratase/3-OH-acyl-CoA dehydrogenase. PBE, peroxisomal bifunctional enzyme; TMTDA, 4,8,12-trimethyltridecanoic acid.

abnormalities were interpreted to indicate a dual effect on phytanic acid catabolism. First, excessive phytanic acid accumulation may have resulted from a partial block in peroxisomal uptake or transport of phytanoyl-CoA. Second, defective thiolytic cleavage of 3-ketopristanoyl-CoA may have led to inhibition of the last step in the β-oxidation pathway. Whereas the first effect seemed to relate to absence of the phytanoyl-CoA carrier function, that could be shown for SCP2 in vitro [Seedorf et al., 1998], the second seemed to be due to absence of the 3-ketopristanoyl-CoA thiolase activity associated with SCPx [Wanders et al., 1997]. Such a mechanism appeared compelling, because a cooperative role of SCP2 and SCPx in metabolism would provide a functional reason for the gene fusion between the two Scp2-encoded functions. Moreover, purified recombinant rat SCP2 protein showed much higher affinity for binding of phytanoyl-CoA than of pristanoyl-CoA, phytanic acid, pristanic acid, or cholesterol and the K_d value was within a physiologically meaningful range (250 nM) [Seedorf et al., 1998].

More recent data from our laboratory support that combined SCP2- and SCPx-deficiency also affects β-oxidation of the cholesterol side chain in bile acid synthesis [Seedorf, 1999]. Upon bile acid synthesis, 3α,7α,12α-trihydroxycholestanoyl-CoA, which is formed in the cytoplasm, must be imported into peroxisomes followed by removal of propionyl-CoA from the side chain in one cycle of peroxisomal β-oxidation [reviewed by Björkhem and Boberg, 1995]. Although homozygous KO mice produced considerable amounts of all primary bile acids, two additional bile species were present in bile from homozygous transgenes but were nondetectable in controls. Moreover, cholesterol-7α-hydroxylase gene expression was at least threefold up-regulated in Scp2(−/−) mice, which could account for partial compensation of the defect. When Scp2(−/−) mice were exposed to a diet containing the bile acid sequestrating drug cholestyramine, known to stress the bile acid synthetic pathway, both unusual bile compounds increased pronouncedly whereas cholic acid remained at a lower level. The mass spectra that were obtained for both compounds pointed to normal 3α,7α,12α-hydroxylated ring structures but abnormal side chains. Comparison with a number of chemically synthesized standards led to their identification as 23-norcholic acid (23-NCA) and 3α,7α,12α-trihydroxy-27-nor-cholestane-24-one. The presence of a 24-keto function in a molecule that was detected in extremely high amounts in bile from cholestyramine challenged Scp2(−/−) mice, suggested very strongly a mechanism involving inefficient thiolytic cleavage of 3α,7α,12α-trihydroxy-24-ketocholestanoyl-CoA, which is the substrate for the thiolase reaction in peroxisomal β-oxidation of the cholesterol side chain. Recently, it could be shown in vitro that the thiolase that is contained within the rat SCPx protein has high 3α,7α,12α-trihydroxy-24-ketocholestanoyl-CoA activity, whereas the conventional peroxisomal thiolase has not [Wanders et al., 1998]. Therefore, it may be assumed that the respective β-keto acid (3α,7α,12α-trihydroxy-24-ketocholestanoic acid) is subject to spontaneous decarboxylation, which would explain the high accumulation of 3α,7α,12α-trihydroxy-27-norcholestane-24-one in Scp2(−/−)mice. By contrast, accumulation of 23-NCA is less clear. Excessive accumulation of this unusual bile acid is known to occur also in the human disease of cerebrotendinous xanthomatosis (CTX) in which defective 27-hydroxylation leads to a block in normal side-chain shortening. Thus, the compound may result from activation of alternative side-chain oxidation pathways that lead to 23-hydroxylation and subsequent production of 23-NCA.

THE ACYL-COA OXIDASE AND X-LINKED ADRENOLEUKODYSTROPHY KNOCKOUT MOUSE MODELS

The first step of peroxisomal β-oxidation of VLCFA is catalyzed by acyl-CoA oxidase (ACO), which converts fatty acyl-CoA to 2-*trans*-enoyl-CoA. Mice deficient in ACO exhibited increased levels of VLCFA, particularly after stress with VLCFA enriched diets [Fan et al., 1996]. The block in peroxisomal β-oxidation of VLCFA was associated with steatohepatitis, increased hepatic H_2O_2 levels, and hepatocellular regeneration, leading to a complete reversal of fatty change by 6 to 8 months of age. The liver of ACO knockout mice displayed profound generalized spontaneous peroxisome proliferation and increased mRNA levels of genes that are regulated by peroxisome proliferator-activated receptor-α (PPARα). Hepatic adenomas and carcinomas developed in ACO−/− mice by 15 months of age, probably as a result of sustained activation of PPARα [Fan et al., 1998]. These observations implicated acyl-CoA or other puta-

tive substrates for ACO as biological ligands for PPARα.

Many of the pleiotropic effects that resulted from the ACO gene disruption resembled those which were present in Scp2(−/−) mice, also showing spontaneous peroxisome proliferation and evidence of chronic PPARα activation. By contrast, steatohepatitis was virtually absent in the SCP2/SCPx-deficient strain and whether the Scp2 gene disruption leads to induction of hepatocarcinogenesis remains to be established. It could be shown, however, that phytanic acid serum concentrations correlated well with expression of PPARα target genes in Scp2(−/−) mice. Moreover, the phytanic acid-induced pleiotropic effects on peroxisome proliferation and lipid metabolism could be mimicked with the PPARα agonist bezafibrate, but not with the RXRα agonist 9-*cis*-retinoic acid. The findings in vivo corresponded to high-affinity binding of phytanic acid to a fused glutathione-S-transferase-murine-PPARα ligand binding domain. It appeared interesting to us that this binding exhibited almost the same affinity as the strong artificial PPARα agonist WY 14,643. In addition, phytanic acid induced expression activation of a PPRE-driven reporter gene in vitro [Ellinghaus et al. 1999]. Taken together, these data provided strong support for phytanic acid, acting as signal involved in direct stimulation of PPARα. This is particularly noteworthy, since direct binding or activation of rodent PPARα could so far not be demonstrated for any other natural substrate of peroxisomal metabolism.

In ACO−/− mice, ACO deficiency imposes a block on VLCFA-CoA to enter the β-oxidation pathway. It is conceivable that unmetabolized VLCFA-CoA may function as biological ligands of PPARα/RXRα, leading to sustained transcriptional enhancement of genes with PPRE-containing promoters in this system. Long-chain acyl-CoAs were once considered to represent a metabolic message responsible for the induction of the β-oxidation system [Berge and Aarsland, 1985; Gottlicher et al., 1993]. This raises the issue whether free fatty acids and unmetabolized synthetic peroxisome proliferators can act as direct ligands of PPARα in vivo or whether activation of this receptor may be mediated by their CoA esters or downstream derivatives resulting from their β-oxidation. It is known that sulfur-substituted fatty acid derivatives and peroxisome proliferators of the fibrate class are activated to their esters with CoA. Although these cannot enter the β-oxidation spiral, they could still function efficiently as peroxisome proliferators in vivo, implying that β-oxidation is not essential to generate the PPARα agonists [Gottlicher et al., 1993; Aarsland and Berge, 1991]. By contrast, there is progressive VLCFA accumulation in X-linked adrenoleukodystrophy (X-ALD; a peroxisomal disorder with impaired VLCFA metabolism, associated with neurological abnormalities and death during childhood) [Lazarow and Moser, 1995]. Despite pronounced VLCFA accumulation, there is no evidence, however, for spontaneous peroxisome proliferation in liver parenchymal cells in X-ALD patients [Lazarow and Moser, 1995; Mosser et al., 1993]. This lack of peroxisome proliferation may not be attributable entirely to differences in the sensitivity of human PPARα [Tugwood et al., 1996], because there is also no indication of the occurrence of spontaneous peroxisome proliferation in mouse models for this disease, developed recently by inactivating the X-ALD gene [Lu et al., 1997; Kobayashi et al., 1997]. In addition, the poor affinity of free VLCFA for binding to recombinant PPARα and the failure to induce peroxisome proliferation by dietary lipid overload [Forman et al., 1997; Ren et al., 1997], leading to increased VLCFA levels [Lazarow and Moser, 1995; Mosser et al., 1993; Lu et al., 1997; Kobayashi et al., 1997] implies that, under in vivo conditions, the free VLCFAs are not effective inducers of PPARα. The remarkable induction of spontaneous peroxisome proliferative response in ACO null mice raises the possibility that the PPARα signal-transducing event is immediately distal to the acyl-CoA synthase-catalyzed fatty acid activation step. However, several long-chain acyl-CoAs did neither bind to recombinant PPARα nor induced PPARα activation in vitro [Forman et al., 1997]. Thus, the factors that mediate peroxisome proliferation in ACO−/− mice remain unclear. One possibility is that PPARα activation is mediated by a still unknown PPARα ligand that is β-oxidized within the peroxisome [Furstenberger et al., 1991]. Such an endogenous ligand of PPARα may potentially contribute to enhanced PPARα activation in the liver of ACO−/− mice. Despite the fact that phytanic acid can be regarded as a bona fide PPARα agonist in mice, the involvement of so far unknown endogenous ligands that accumulate along with phytanic acid in

Scp2(−/−) mice and signal PPARα activation even more effectively cannot be ruled out.

Nonetheless, the data establish clearly that both genes, ACO and Scp2, are required for efficient peroxisomal oxidation of certain fatty acids and at the same time they are key regulators of PPARα function in vivo. These mouse models may therefore provide tools in the search for so far unknown natural PPARα agonists and in screening for in vivo antagonists for this receptor.

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