Regulation of sterol carrier protein gene expression by the Forkhead transcription factor FOXO3a

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Abstract The *SCP* gene encodes two proteins, sterol carrier protein X (SCPx) and SCP2, that are independently regulated by separate promoters. SCPx has been shown to be the thiolase involved in the breakdown of branched-chain fatty acids and in the biosynthesis of bile acids. The in vivo function of SCP2 however remains to be established. The transcriptional regulation of SCPx and SCP2 is unclear, but their promoter regions contain several putative regulatory domains. We show here that both SCPx and SCP2 are up-regulated by the daf-16-like Forkhead transcription factor FOXO3a (also known as FKHRL1) on the level of promoter activity. It was recently described that Forkheads regulate protection against (oxidative) stress in both *Caenorhabditis elegans* and mammalian cells. We looked into a role for SCP2 in the cellular defense against oxidative damage and found that a fluorescent fatty acid analog bound to SCP2 is protected against H_2O_2/Cu^{2+}-induced oxidative damage. ▮▮ We propose a model for the way in which SCP2 could protect fatty acids from peroxidation.—Dansen, T. B., G. J. P. L. Kops, S. Denis, N. Jelluma, R. J. A. Wanders, J. L. Bos, B. M. T. Burgering, and K. W. A. Wirtz. **Regulation of sterol carrier protein gene expression by the Forkhead transcription factor FOXO3a.** *J. Lipid Res.* 2004. **45:** 81–88.

Supplementary key words oxidative stress • aging • peroxisome • fatty acid oxidation

Sterol carrier protein X (SCPx) and SCP2 are encoded by the *scp* gene. This gene contains two promoters, one upstream of the gene and a second one within intron XI (1). Transcription from either promoter yields two transcripts, probably due to alternative polyadenylation (2). When started from the first promoter, transcripts of 2.4 kb and 3.0 kb are formed, while the second promoter gives rise to 1.1 and 1.7 kb transcripts. Both the 2.4 kb and the 3.0 kb mRNAs encode the full-length 58 kDa SCPx,

whereas the 1.1 and 1.7 kb mRNAs encode the 15 kDa preSCP2. Additionally, SCP2 can be formed by cleavage of SCPx, yielding SCP2 and a 46 kDa protein (3). Both SCPx and the 46 kDa protein have 3-oxoacyl CoA thiolase activity catalyzing the thiolytic step in both the peroxisomal breakdown of branched-chain fatty acids (BCFAs) such as pristanic acid, and in the formation of the CoA-esters of cholic acid and chenodeoxycholic acid from di- and trihydroxycholestanoic acid (4, 5). The C terminus contains a type I peroxisomal targeting signal (AKL in human, rat, mouse, rabbit, and chicken) that directs SCPx/SCP2 to peroxisomes. SCP2 has been shown to have in vitro lipid transfer activity (6) and to bind very long chain fatty acid- and PUFA-CoA esters with high affinity (7, 8). Moreover SCP2 has been implicated in cholesterol trafficking and metabolism (9), although *scp* null mice show no obvious defects in their cholesterol homeostasis (10). Given the absence of SCPx, these mice are impaired in the β-oxidation of BCFAs and in bile-acid formation (11).

It was previously demonstrated that the promoters for SCPx and SCP2 are differentially regulated. Sequence analysis showed that the promoter region for SCP2 contains several regulatory domains, including a steroidogenic factor-1 binding motif, activator protein-1 elements, an insulin response element, and a peroxisomal proliferator response element [reviewed in ref. (9)]. Transcription of *scp* was shown to be induced upon treatment with the protein kinase A agonist 8-bromo-cAMP (12). Treatment of cells with a peroxisome proliferator did not affect *scp* expres-

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A

WB: anti-SCPx-thiolase

DL23   DLD-1

58 kDa SCPx
46 kDa SCPx-thiolase

hrs 4OHT   0   4   8   12   16   24   0   24

B

WB: anti-SCP2

SCP2

WB: anti-thiolase

straight-chain-thiolase

WB: anti-acyl-CoA oxidase

70 kDa
acyl-CoA oxidase
50 kDa

DL23
hrs 4OHT   0   8   16   24

C

relative specific activity (+4OHT/-4OHT)

D-PBE   SCPx
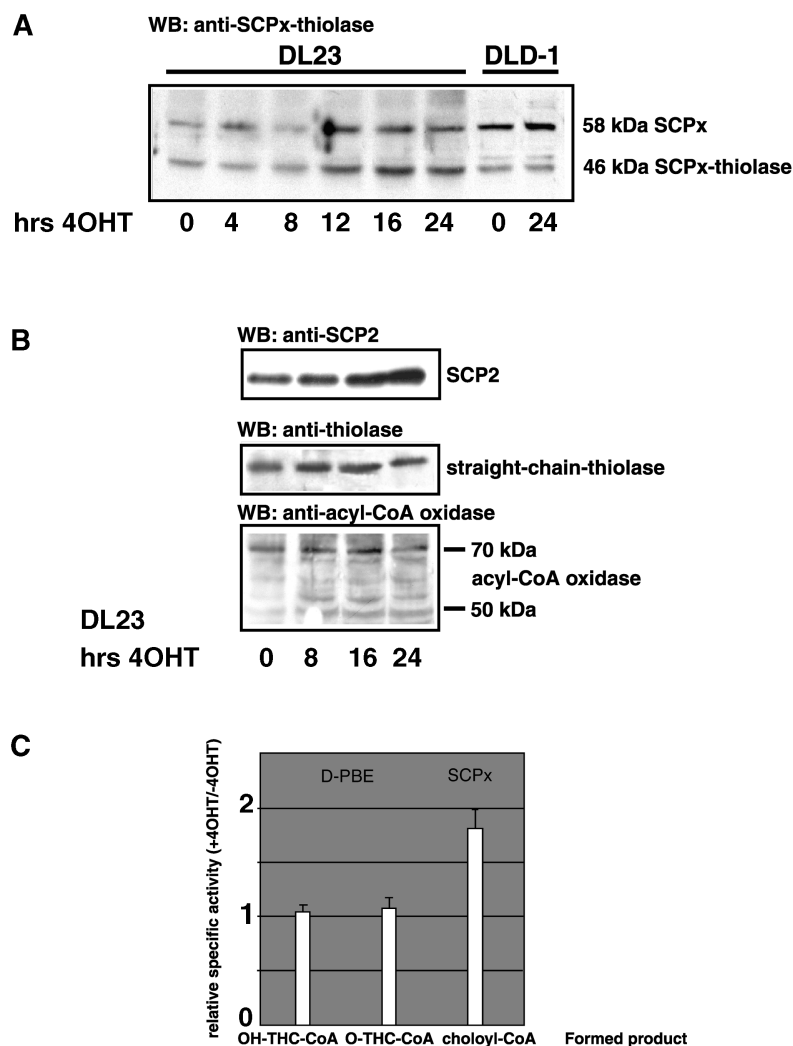
OH-THC-CoA   O-THC-CoA   choloyl-CoA   Formed product

Fig. 1. Sterol carrier protein X (SCPx)/SCP2 protein levels are increased upon induction of FOXO3a. A: The DL23 cell line expresses FOXO3a-estrogen receptor (ER), the activity of which is fully dependent on 4-hydroxy-tamoxifen (4OHT). Treatment of this cell line with 4OHT resulted in an increase of protein levels of SCPx, the 46 kDa fragment of SCPx, and SCP2 (also see B). A control cell line, DLD-1 that is not expressing the FOXO3a-ER construct shows no increase in SCPx/SCP2 protein levels upon treatment with 4OHT. B: Acyl-CoA oxidase and thiolase protein levels are unaffected by treatment with 4OHT. The actin levels of these samples remained unchanged and have been published previously (23). C: Treatment with 4OHT led to a 2-fold increase of SCPx thiolase activity as monitored by the formation of choloyl-CoA from 24-ketotrihydroxycholestanoyl-CoA. The D-peroxisomal bifunctional enzyme (D-PBE) activity measured as the formation of 24-hydroxy- and 24-keto-trihydroxycholestanoyl-CoA (24-OH-THC-CoA and 24-keto-THC-CoA) remained unchanged (n = 3). Western blots were repeated at least three times and are typical results. Error bars indicate SD.

sion (13), nor was *scp* expression enhanced in the livers of clofibrate-treated rats (14), although recently Lopez, Irby, and McLean (15) showed that SCPx promoter activity is regulated through PPARα in rats. Decreased levels of SCP2 were described in *ras*- and *src*-transformed colon cells (16). Similarly, hepatoma cells express less SCPx/SCP2, as compared with hepatocytes (17). This could be indicative of a link between SCPx/SCP2 expression and tumorigenesis and/or growth factor signaling. However, both active Ras and Src couple to a large variety of signaling pathways that can regulate gene transcription. One of these pathways, the Age-1/PKB/DAF-16 pathway (18, 19) has been shown in *Caenorhabditis elegans* to control dauer formation. One of the characteristics of dauer formation is an increased resistance against oxidative damage and altered metabolism (18, 20, 21). The human homologues of the daf-16 Forkhead transcription factor (FOXO1, FOXO4, and FOXO3a, also known as FKHR, AFX, and FKHRL1, respectively) have been implicated in cell cycle control and, in hematopoietic cells, in apoptosis (22). Additionally, it was shown that human homologs of the daf-16-like Forkhead transcription factors protect against oxidative stress by increasing the levels of the antioxidant enzymes MnSOD and catalase and the DNA repair enzyme GADD45

(23–25). It has been suggested that the shift from glucose to lipid metabolism upon activation of daf-16 in *C. elegans* is also present in mammalian cells upon activation of the FOXO transcription factors (22). This hypothesis is strengthened by the findings that: *1*) FOXO3a activation positively regulates the levels of catalase involved in detoxification of H_2O_2 derived from peroxisomal β-oxidation of fatty acids in mice (25); *2*) FOXO3a regulates the expression of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase involved in the formation of ketone bodies that can serve as metabolite, preserving limited glucose for use by the brain (26), in HepG2 cells; and *3*) FOXO1 upregulates the expression of lipoprotein lipase in skeletal muscle, resulting in enhanced hydrolysis of fatty acids from triglycerides, which are then further utilized for β-oxidation (27). The daf-16 binding element (DBE) to which daf-16-like Forkhead transcription factors bind was identified as 5'-TTGTTTAC-3' (28). We found by in silico screening that the human *scp* gene contains several near-perfect DBEs (5'-TTGTTTAT-3') at positions −4,696, +451, and +2,832. The +2,832 site is within the intron containing the preSCP2 promoter. The −3,473 and +2,832 sites also fully conform to the sequence required for binding Forkhead family members, TRTTTAY (29). The promoters for
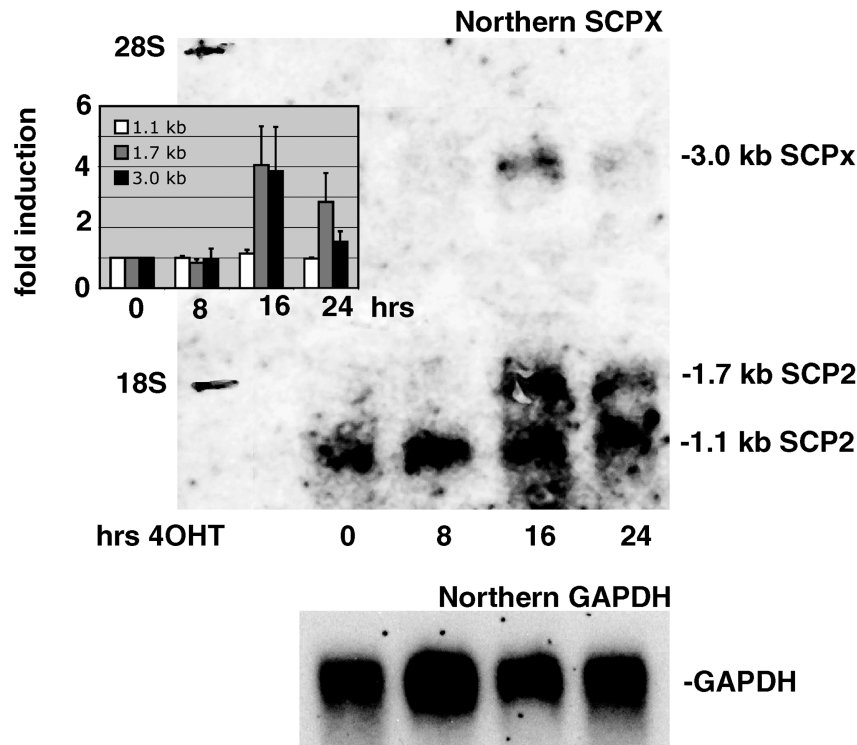
DL23 total mRNA

Fig. 2. SCPx/SCP2 mRNAs are increased upon induction of FOXO3a. Treatment of the DL23 cell line with 4OHT for up to 24 h led to a marked increase in the 1.7 kb mRNA of preSCP2, whereas the 1.1 kb messenger remained unchanged. The 3.0 kb SCPx messenger was also induced, reaching a maximum at 16 h. The 2.4 kb SCPx mRNA was not detected in these cells. Inset: normalized average increases of SCPx/SCP2 mRNAs (n = 3; error bars indicate SD). Quantities were determined by densitometry and normalized to GAPDH mRNAs and t = 0 h.

the mouse *scp* gene have not been characterized fully, but the 5′ region of this gene as well as intron XI contained several putative DBEs as found by screening the mouse genome database at the Celera Discovery System website.

We will demonstrate that both SCPx and SCP2 are regulated by daf-16-like Forkhead transcription factors at the level of promoter activity. This result is corroborated by an increase in mRNA, protein and SCPx thiolase activity. In accordance with one of the roles of Forkheads in both humans and *C. elegans*, i.e., upregulation of antioxidant capacity, SCP2 was able to protect a fluorescent fatty acid analog from oxidation by H_2O_2/Cu^{2+}, suggesting a role for SCP2 in the protection of fatty acids against peroxidation.

EXPERIMENTAL PROCEDURES

Materials

Culture media were from Gibco-Life Technologies; other chemicals were from Sigma, unless stated otherwise.

Tissue culture and cell lines

The DL23 cell line was created as follows (30): linearized pcDNA3-HA-FOXO3a.A3-estrogen receptor (ER) was transfected into DLD-1 human colon carcinoma cells by electroporation. Transfectants were selected for 2 weeks on 500 μg/ml geneticin.

Subsequently, clones were isolated and analyzed for expression of the fusion protein. The DL23 subclone was chosen for further study. The DLD-1 and DL23 cell lines were maintained in RPMI-1640 with standard supplements and the appropriate selection antibiotic. 4-Hydroxy-tamoxifen (4OHT) was administered at a concentration of 500 nM. Rat-1 fibroblasts, as well as C2C12, 3T3L6, and human embryonic kidney (HEK) 293 cells, were cultured in bicarbonate-buffered DMEM under 5% CO_2 atmosphere.

Northern blotting

Two micrograms of mRNA (polyA-Tract, Promega) purified from 1 mg total RNA (RNAZol, TEL-TEST, Inc.) was run on a formaldehyde denaturing gel and blotted onto GeneScreen-Plus nylon membrane (NEN). The blots were hybridized using ^{32}P-labeled probes representative for SCPx and for GAPDH (NotI-linearized pUC19-GAPDH) probes. These probes for SCPx were the *BamH*I and *Xho*I fragments of pEYFP-C1-SCPx, which was made by inserting the cDNA encoding SCPx (a kind gift from Dr. S. Ferdinandusse) into the multiple cloning site of pEYFP-C1 (Clontech).

Western blotting

SDS-PAGE and Western blotting were performed as described (23, 30). Antibodies against the N-terminal thiolase segment of SCPx, SCP2, acyl-CoA oxidase and straight-chain thiolase were as described previously (31, 32).

Enzyme activity measurements

The activities of D-peroxisomal bifunctional enzyme (D-PBE) and SCPx were measured as described previously (33) using 3α,
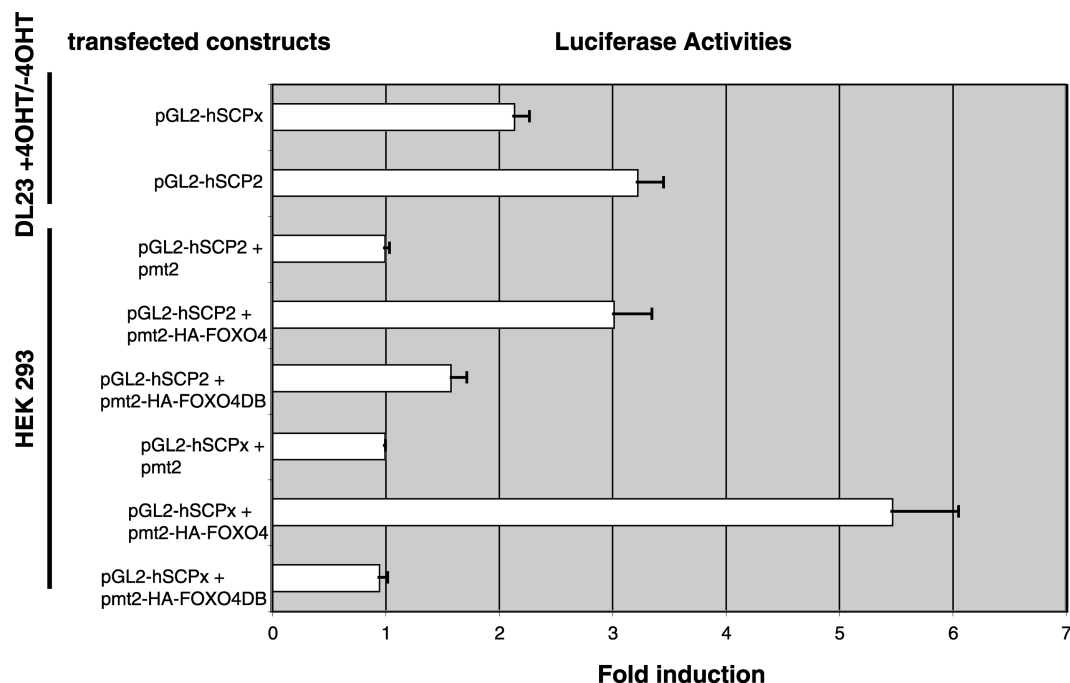
Fig. 3. hSCPx and hSCP2 promoter activities were increased by FOXO3a and FOXO4. DL23 cells were transfected with the above luciferase constructs. Cells were then split, half of the cells were treated with 4OHT for 16 h, and luciferase activity was measured. The graph displays fold increase in luciferase activity of +4OHT over −4OHT. Human embryonic kidney 293 fibroblasts, which have a high transfection efficiency, were transfected with luciferase constructs containing either the hSCP2 or the hSCPx promoter, in combination with pMT2-HA-FOXO4, pMT2-HA-FOXO4-DB (impaired in its binding to the daf-16 binding element), or the vector control pMT2 alone. Luciferase activity was measured and normalized to the activities measured in the samples in which pGL2-hSCP2 and pGL2-hSCPx, respectively, were cotransfected with pMT2. All luciferase experiments were repeated at least three times. Error bars indicate SD.

7α,12α-trihydroxy-5β-cholest-24-en-26-oyl-CoA (24(E)-ene-THC-CoA) and 24-keto-3α,7α,12α-trihydroxy-5β-cholest-26-oyl-CoA as specific substrates for D-PBE and SCPx, respectively, followed by HPLC analysis to resolve the different CoA-esters produced. Lysates of DL23 cells that were either untreated or treated with 4OHT for 24 h were compared.

Luciferase assay

pGL2-vectors containing the hSCPx and hSCP2 promoters followed by luciferase were a kind gift of Dr. J. Strauss III. HEK 293 cells, which have a high transfection efficiency, were cotransfected with a pMT2-HA-FOXO4 and either pGL2-hSCPx or pGL2-hSCP2. As a control, pMT2-HA-FOXO4-DB expressing FOXO4 with an impaired binding domain or empty pMT2 vector was used. The luciferase assay in HEK 293 cells, as well as the used FOXO4 and FOXO4-DB constructs, were previously described (34). DL23 cells were transfected with the above pGL2-hSCP2 and hSCPx vectors. After recovery, they were split, and half of them were treated with 4OHT for 16 h, followed by measurement of the luciferase activity.

Antioxidant capacity assay for preSCP2

To investigate whether preSCP2 could protect the fatty acid analog C11-BODIPY [581/591] (Molecular Probes) against oxidation by the hydroxyl radical-inducing system H_2O_2/Cu^{2+} (35), we developed the following assay. Recombinant rat preSCP2 was overexpressed and purified as described by Ossendorp, Geijtenbeek, and Wirtz (36). First, we checked whether preSCP2 could bind C11-BODIPY[581/591] by loading preSCP2 with Pyrene-C12 (PyrC12, Molecular Probes), which is a good fluorescence resonance en-

ergy transfer (FRET) acceptor when the single Trp residue in the preSCP2 binding site is excited at 280 nm. FRET efficiency between Trp and PyrC12 is a good indicator for binding (7). The decay of FRET upon addition of C11-BODIPY[581/591] to the preSCP2/PyrC12 complex is indicative of competition between the two fluorescent fatty acids. Equimolar concentrations of PyrC12
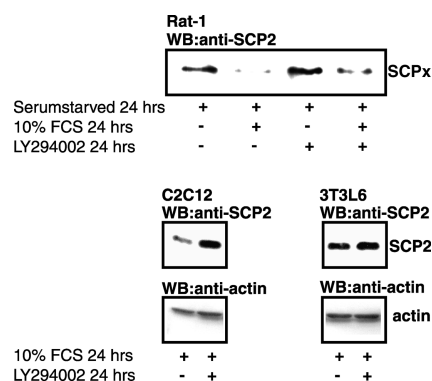

Fig. 4. Cell cycle-dependent expression of SCPx/SCP2. Serum-starved Rat-1 fibroblasts have higher protein levels of SCPx, as compared with Rat-1 fibroblasts that were stimulated for 24 h with serum. Treatment with the PI3K inhibitor LY294002 could partially prevent the decrease of SCPx upon treament with serum. Similar results were obtained for SCP2. Other cell lines tried, C2C12 cells and 3T3L6 cells, also showed a negative regulation of SCP2 by PI3K, as judged by treating cycling cells with LY294002.

and C11-BODIPY$^{581/591}$ resulted in a FRET decay of ~35%, which means that the affinity for C11-BODIPY$^{581/591}$ is ~1.5 times less than for PyrC12, which has a K_A of 0.24 μM.

C11-BODIPY$^{581/591}$ dissolved in ethanol was added to PBS to a final concentration of 0.5 μM (5 μl in 1 ml PBS). The fluorescence of the oxidized (green, Ex 490 Em 510 nm) and the intact (red, Ex 570 Em 590 nm) fluorophores were acquired using a PTI fluorimeter. Recombinant human preSCP2 purified from *Escherichia coli* was added to a final concentration of 0.5 μM. After equilibration of the signals, H_2O_2 (200 μM) was added, and 1 min later, Cu^{2+} (40 nM), a catalyst for hydroxyl radical formation from H_2O_2. The green and red signals were acquired for 25 min, and afterwards, the fraction oxidized C11-BODIPY$^{581/591}$ was calculated in each time point by dividing the green signal by the sum of the green and red signals. The same experiment was performed using BSA as a control. Addition of a molar excess of preSCP2 did not increase the protection, indicating that all C11-BODIPY$^{581/591}$ was bound to preSCP2 under these experimental conditions. This was also true for BSA.

RESULTS

The Forkhead transcription factor FOXO3a regulates *scp* expression

To study the possible involvement of Forkheads in the regulation of SCPx/SCP2 protein levels, we made use of a DL23 cell line expressing an HA-FOXO3a.A3-ER fusion protein. This protein is constitutively expressed but is inactive unless presented with a modified ligand for the ER, 4OHT (30). Treatment of the DL23 subclone with 500 nM 4OHT for 24 h resulted in the specific activation of FOXO3a as measured by previously described Forkhead activity assays (37). This activation was not observed in the control cell line, DLD-1. Treatment of DL23 cells with 500 nM 4OHT for 0 h, 4 h, 8 h, 12 h, 16 h, and 24 h resulted in a time-dependent increase of SCPx, 46 kDa protein, and SCP2 protein levels (**Fig. 1A, B**). Acyl-CoA oxidase and thiolase protein levels were not increased (Fig. 1B). Actin levels for these blots have been described previously (23) and were equal over the samples. Failure of 4OHT to alter SCPx/SCP2 protein levels in the parent DLD-1 line demonstrates a specific requirement for FOXO3a-ER expression (Fig. 1A).

To determine whether the increased protein levels of SCPx were functional, we determined the activity of SCPx by measuring the SCPx-associated thiolase activity using 24-keto-THC-CoA as specific substrate (4, 5). Furthermore, we determined the activity of D-PBE, which is the enzyme with both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activity, which preceeds SCPx (4, 5). As shown in Fig. 1C, the specific activity of SCPx increased ~2-fold upon 4OHT treatment, whereas no effect was observed on D-PBE.
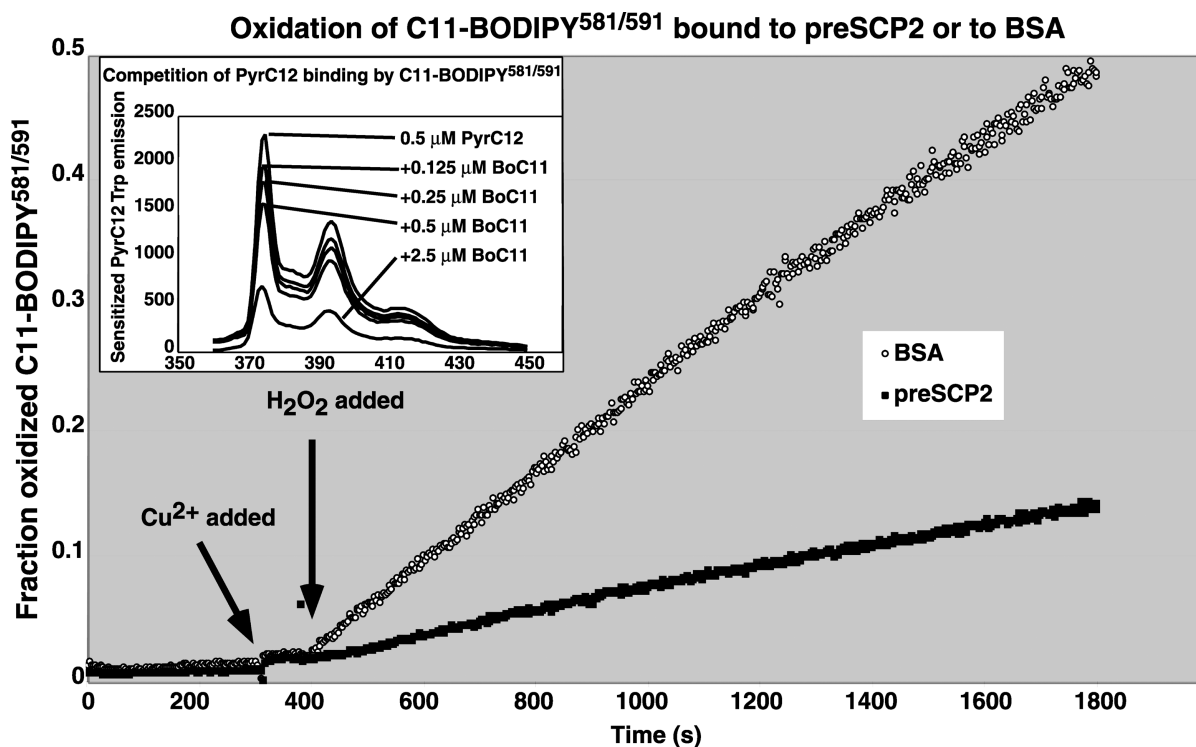

Oxidation of C11-BODIPY$^{581/591}$ bound to preSCP2 or to BSA

Fig. 5. SCP2 protects against radical attack. C11-BODIPY$^{581/591}$ bound to preSCP2 is better protected from oxidation by hydroxyl radicals formed by H_2O_2/Cu^{2+} than C11-BODIPY$^{581/591}$ bound to BSA. PreSCP2 or BSA and C11-BODIPY$^{581/591}$ were added in equimolar amounts. The Fenton reaction catalyst copper (40 nM) was added, followed by H_2O_2 (200 μM). The green (oxidized form) fluorescence and red (intact form) fluorescence were monitored and plotted versus time as the fraction oxidized, which is the green fluorescence intensity, divided by the total fluorescence intensity. The curves shown are representative of four independent experiments. Binding of C11-BODIPY$^{581/591}$ to preSCP2 was checked using a fluorescence resonance energy transfer (FRET)-based competition assay (insert; BoC11 = C11-BODIPY$^{581/591}$). preSCP2 was complexed with an equimolar amount of PyrC12. The Trp residue in the binding site was excited at 280 nm, and the sensitized pyrene emission spectrum was measured at 370–450 nm. Pyrene was competed out of the binding site by C11-BODIPY$^{581/591}$, as indicated by the decay in FRET efficiency.

Northern blotting of total mRNA isolated from untreated DL23 cells revealed that these cells express mainly the 1.1 kb *scp* transcript; a very weak signal was found for the 1.7 kb and 3.0 kb transcripts. Treatment with 4OHT for 24 h led to an increase of the 1.7 and 3.0 kb transcripts (reaching a maximum at 16 h), whereas the levels of 1.1 kb messenger remained unchanged (see **Fig. 2**). The 2.4 kb transcript was not detectable in this cell line.

Luciferase assays showed that treatment of the DL23 subclone with 4OHT led to an increased promoter activity of hSCP2 and hSCPx. To show that this effect was general and specific for daf-16-like Forkhead transcription factors, we performed an experiment in HEK293 cells, which have a high transfection efficiency. Expression of FOXO4, a FOXO3a homolog that binds to the same DNA domain, increased promoter activity of both hSCP2 and hSCPx. Expression of FOXO4-DB, impaired in its binding to the DBE, affected promoter activity to a much lesser extent (**Fig. 3**).

Cell cycle-dependent expression of SCPx/SCP2

Because SCPx/SCP2 levels are low in cancers, we studied whether growth factor signaling contributes to their regulation. Serum starvation has been shown to decrease the amount of phosphorylated PKB, and, as a result, leads to a more active Forkhead transcription factor (23). In agreement with this, serum-starved Rat1 fibroblasts showed high levels of SCPx/SCP2 protein, as compared with cells that were exposed to fetal calf serum (**Fig. 4**). Treatment of the serum-starved cells with the PI3K inhibitor LY294002 showed levels of SCPx/SCP2 similar to those without LY294002. However, this inhibitor limited the observed decrease of SCPx/SCP2 upon overnight stimulation of the cells with serum; the amount of SCPx/SCP2 protein remaining was about twice as high as that in untreated cells. This is indicative of a role for PI3K signaling in the negative regulation of SCPx/SCP2 protein levels. Similar results were obtained in two other cell lines, C2C12 and 3T3L6. This suggests that the PI3-K-dependent regulation of SCPx/SCP2 is a general phenomenon.

SCP2 protects against lipid peroxidation

Both preSCP2 and SCP2 can bind fatty acyl-CoA esters with high affinity (7, 8). Here we used preSCP2 to determine whether this protein is able to protect C11-BODIPY[581/591] from oxidation by hydroxyl radicals. The accommodation of the fatty acid analog C11-BODIPY[581/591] into the binding site of preSCP2 was assessed by a FRET-based competition assay (see inset of **Fig. 5**) as described (7). As a control, we used the fatty acid carrier BSA. As shown in Fig. 5, preSCP2 protected the fatty acid analog four times more efficiently than an equimolar amount of BSA. Taking into account that BSA has five high-affinity fatty acid binding (38) sites as compared with one for preSCP2 (39), the capacity of preSCP2 to protect fatty acids against oxidation is much greater than that of BSA.

DISCUSSION

Peroxisomes produce large amounts of H_2O_2 and are therefore sensitive to oxidative stress. To protect the peroxisomes from this stress, a battery of antioxidant enzymes is present, including catalase, superoxide dismutases, and glutathione peroxidase (40, 41). Furthermore, antioxidants such as glutathione and ascorbate have been detected in the peroxisomal matrix (42). On the other hand, H_2O_2 formed in the peroxisome may leak out into the cytosol (41), indicating that the antioxidant capacity of the peroxisome is not necessarily sufficient. Hence, it may well be that H_2O_2 escaping from the scavenging system is converted into hydroxyl radicals in the presence of metal^{2+} ions. In support of this, lipid peroxidation events have been reported to occur at the peroxisomal membrane (43). Fatty acids that are susceptible to radical-induced oxidation include PUFAs and intermediates of the fatty acid β-oxidation cycle. In the first step of β-oxidation, different acyl-CoA oxidases introduce a double bond at the β-position of fatty acyl-CoA esters while generating H_2O_2. SCP2 was shown to be complexed to acyl-CoA oxidase in the peroxisome in mammalian cells (32). We show here that SCP2, in addition to its ability to bind unsaturated fatty acyl-CoA esters with high affinity, protects the fatty acid analog C11-BODIPY[581/591] from oxidation by hydroxyl radicals. We therefore speculate that the association of SCP2 with the β-oxidation complex could be important for the protection of unsaturated fatty acid intermediates that are leaving the acyl-CoA oxidase active site against attack
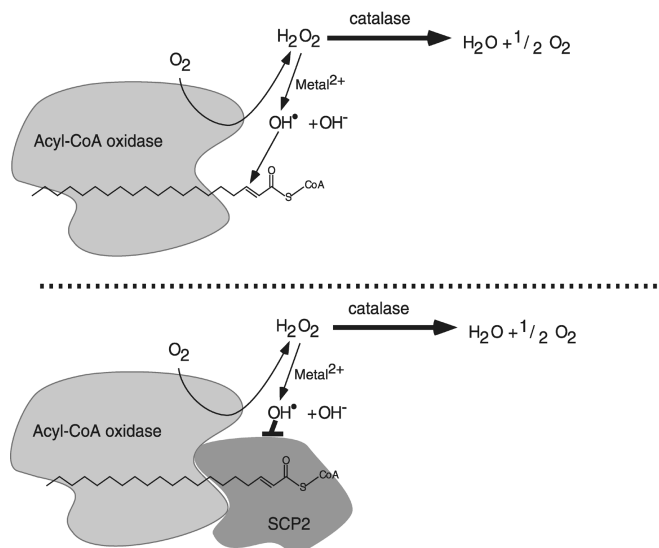

Fig. 6. Model for the protective role of SCP2. We propose that SCP2 protects fatty acids with a 2-enoyl bond that are susceptible to oxidation by hydroxyl radicals. Acyl-CoA oxidase, which introduces the 2-enoyl bond, also generates hydrogen peroxide that can be interconverted to hydroxyl radicals in the Fenton reaction. Formation of a complex of acyl-CoA oxidase with SCP2 could serve to shield the fatty acids that are leaving (or entering) the acyl-CoA oxidase reactive site from damage by hydroxyl radicals. The D-PBE contains a domain that is very homologous to SCP2, perhaps reflecting a similar function.

by radicals (**Fig. 6**). It is to be noted that C11-BODIPY[581/591] does not have the exact same structure as a fatty acyl-CoA ester (although quite similar), but previous studies showed that the sensitivity of C11-BODIPY[581/591] toward oxidation is comparable to that of natural PUFAs (44). The D-PBE has a domain that is homologous to SCP2 and may serve a similar purpose. Notably, SCP2 does not contain tyrosine residues; this amino acid was shown to be susceptible to forming *o,o*-dityrosine bonds under conditions of oxidative stress (45). The lack of tyrosine residues in SCP2 may be an adaptation to its oxidative environment. Niki et al. (46, 47) showed that the yeast SCP2 homolog PXP-18 forms a tight near-stoichiometric complex with acyl-CoA oxidase, thereby protecting it from thermal inactivation at 70°C. This study showed also that PXP-18 protected other peroxisomal enzymes from thermal inactivation, and the authors suggested that PXP-18 might function as a stress protein.

The daf-16-like Forkhead transcription factors have been implicated in the regulation of the cellular antioxidant defense mechanism in both *C. elegans* (18, 19) and humans (23). Here we show that the expression of the *scp* gene products SCPx and SCP2 is positively regulated by the Forkhead transcription factor FOXO3a. We do not know whether this regulation is direct or indirect, but the *scp* gene contains multiple putative binding elements for Forkhead transcription factors. Northern and Western blot analyses show a clear upregulation of SCPx and SCP2 transcript and protein levels by active FOXO3a. Interestingly, the transcripts specifically induced by FOXO3a are the 1.7 kb preSCP2 mRNA and the 3.0 kb SCPx mRNA, which are most probably the longer polyadenylated forms (2). Normally, the 1.7 kb messenger is predominantly expressed in the brain (31), which is also one of the main sites of FOXO3a expression in the adult mouse (28).

In contrast to our findings for SCPx/SCP2, FOXO3a does not upregulate the straight-chain acyl-CoA oxidase, a marker enzyme for peroxisome proliferation, or the peroxisomal straight-chain thiolase. These two enzymes are part of the so-called inducible β-oxidation system and are induced by the PPARs. SCPX/SCP2 expression is not regulated through these transcription factors (14, 16). The inability of FOXO3a to upregulate the classic inducible β-oxidation pathway excludes a general shift to peroxisomal lipid metabolism upon activation of FOXO3a. In support of this, in silico analysis of the promoters of these β-oxidation enzymes revealed no DBEs. In addition, the activity of D-PBE that catalyzes the two steps in the β-oxidation cycle before the thiolytic cleavage by SCPx remains unchanged after induction of the FOXO3a by 4OHT in the DL23 subclone. Although the FOXO transcription factors have been implicated in the regulation of fatty acid metabolism, our data suggest that the Forkhead-induced upregulation of SCPx in the DL23 cells serves a function other than the upregulation of branched fatty acid breakdown. It is to be noted that BCFAs comprise only a small amount of the fatty acids in triglycerides, and perhaps the activity of the noninducible β-oxidation cycle need not be increased in times of increased fatty acid metabolism.

Apart from being the thiolase for BCFAs, SCPx may serve as a precursor of SCP2, contributing to the antioxidant capacity of the peroxisome.

Direct in vivo evidence for an antioxidant function of SCP2 is lacking; however, ongoing studies by our laboratory, in collaboration with the group of Dr. Udo Seedorf, show higher levels of lipid peroxidation products in the livers of SCPX/SCP2 knockout mice, as well as in isolated hepatocytes derived from these mice (unpublished observations). Although this may be due to the prolonged activation of PPARα that has been described in these mice (48), it is tempting to speculate that these cells are directly affected in their ability to protect fatty acids from oxidative damage by reduced free radical scavenging in the absence of SCPX/SCP2.

In summary, we show that SCPX/SCP2 is regulated through the Forkhead transcription factor FOXO3a. We speculate that SCP2 protects (poly)unsaturated fatty acyl-CoA esters and intermediates in the β-oxidation cycle from oxidation by reactive oxygen species in the peroxisome, and that the upregulation of SCPx/SCP2 via FOXO3a is in line with the regulation of antioxidant defense mechanisms in the cell by this transcription factor.∎

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