

A mitochondrial ketogenic enzyme regulates its gene expression by association with the nuclear hormone receptor PPAR α

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Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-CoAS) is a key enzyme in ketogenesis, catalyzing the condensation of acetyl-CoA and acetoacetyl-CoA to generate HMG-CoA, which is eventually converted to ketone bodies. Transcription of the nuclear-encoded gene for mHMG-CoAS is stimulated by peroxisome proliferator-activated receptor (PPAR) α , a fatty acid-activated nuclear hormone receptor. Here we show that the mHMG-CoAS protein physically interacts with PPAR α *in vitro*, and potentiates PPAR α -dependent transcriptional activation via the cognate PPAR response element of the mHMG-CoAS gene *in vivo*. Immunofluorescence of transiently transfected cells demonstrated that in the presence of PPAR α , mHMG-CoAS is translocated into the nucleus. Binding to PPAR α , stimulation of PPAR α activity and nuclear penetration require the integrity of the sequence LXXLL in mHMG-CoAS, a motif known to mediate the interaction between nuclear hormone receptors and coactivators. These findings reveal a novel mechanism of gene regulation whereby the product of a PPAR α -responsive gene, normally resident in the mitochondria, directly interacts with this nuclear hormone receptor to autoregulate its own nuclear transcription.

Keywords: cofactor/HMG-CoA synthase/mitochondria/nuclear hormone receptor/transcription

Introduction

Ketogenesis is a tightly regulated metabolic pathway carried out in the liver that supplies lipid-derived energy to the brain under normal conditions, and to peripheral tissues during starvation and sustained exercise and in disease states such as diabetes (McGarry and Foster, 1980). Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-CoAS) is the first and rate-limiting enzyme in this pathway, catalyzing the condensation of acetyl-CoA and acetoacetyl-CoA in the mitochondria to generate HMG-CoA and free CoA (Quant, 1994). HMG-CoA is eventually converted, through the actions of HMG-CoA lyase and D-3-hydroxybutyrate dehydrogenase, into the ketone bodies acetoacetate and β -hydroxybutyrate, which

are used as sources of oxidative fuels in extrahepatic tissues.

Mitochondrial HMG-CoAS is regulated at both the transcriptional and post-transcriptional level by mechanisms that can increase both the amount and activity of the enzyme (Quant, 1994). Fatty acids have been shown to induce transcription of the nuclear-encoded gene for mHMG-CoAS through activation of the peroxisome proliferator-activated receptor (PPAR) α , a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors (Rodriguez *et al.*, 1994). PPAR α , and the highly related PPAR β and PPAR γ subtypes, regulate the expression of a wide assortment of genes that are important in metabolic homeostasis, cellular differentiation and energy utilization, and the different subtypes have been variously implicated in molecular processes associated with diabetes, obesity, atherosclerosis, thermogenesis and inflammation (Devchand *et al.*, 1996; Lemberger *et al.*, 1996; Schoonjans *et al.*, 1996; Latruffe and Vamecq, 1997; Jiang *et al.*, 1998; Nagy *et al.*, 1998; Puigserver *et al.*, 1998). PPAR α , which is expressed predominantly in liver (Braissant *et al.*, 1996), controls the transcription of genes involved in fatty acid oxidation, fatty acid synthesis and ketogenesis, among other processes, in response to nutritional, hormonal and environmental stimuli. PPAR α activates transcription by binding cooperatively with its obligate heterodimerization partner 9-*cis* retinoic acid receptor (RXR α) to characteristic PPAR response elements (PPREs) present in the promoter regions of target genes (Kliwer *et al.*, 1992; Tugwood *et al.*, 1992; Zhang *et al.*, 1992; Marcus *et al.*, 1993), including the PPRE identified upstream of the rat mHMG-CoAS gene (Rodriguez *et al.*, 1994).

The activity of nuclear hormone receptors such as PPAR is modulated by complex interactive networks of auxiliary receptor-interacting factors that serve as coactivators and corepressors of hormone-responsive pathways (Glass *et al.*, 1997; Shibata *et al.*, 1997). We have been using the yeast two-hybrid system to attempt to identify novel factors that specifically modulate the function of PPAR α . We report here that mHMG-CoAS interacts directly with PPAR α *in vitro* and stimulates transactivation by this receptor *in vivo*. Stimulation of PPAR α activity was only observed via the cognate PPRE present in the mHMG-CoAS gene but not with other PPREs. Moreover, mHMG-CoAS, which is located predominantly in mitochondria, was found to be present in the nucleus when coexpressed with PPAR α . Finally, interaction with PPAR α , stimulation of PPAR α activity and PPAR α -dependent nuclear accumulation were dependent upon a nuclear hormone receptor-cofactor interaction motif, LXXLL, which is present in mHMG-CoAS. Our findings demonstrate that the product of a PPAR α target gene, which functions as a ketogenic enzyme in mitochondria, can also specifically autoregulate

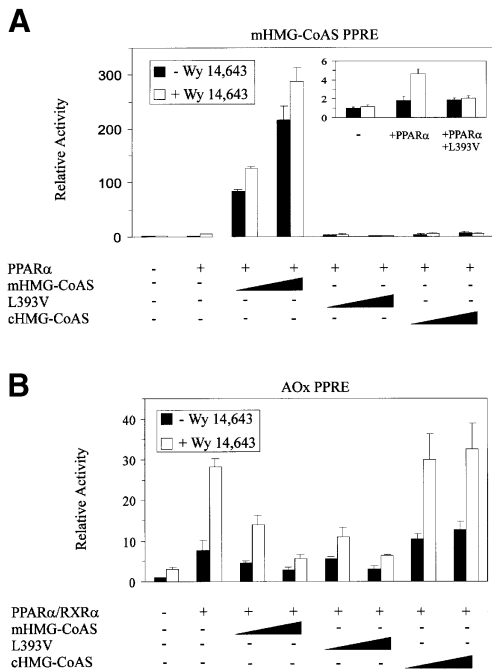


Fig. 2. mHMG-CoAS stimulates transactivation by PPAR α *in vivo*. **(A)** A luciferase reporter gene containing the rat mHMG-CoAS PPRE was cotransfected with expression vectors for PPAR α (1.5 μ g), mHMG-CoAS (1.25 or 2.5 μ g) or L393V (1.25 or 2.5 μ g), in the absence or presence of Wy-14,643, as indicated, and luciferase activity was measured. The values shown are averages (\pm SEM) from at least two separate experiments that were carried out in duplicate, and were normalized to the activity of the reporter gene alone, which was set as 1. The inset is a representation of some results at a different scale to show better the induction of the reporter gene by PPAR α . **(B)** Transfections were carried out as above using a reporter gene that contained the AOX-PPRE. In this case, an expression vector for RXR α (1.5 μ g) was included in the transfections.

ent transfection assays with a reporter gene containing the cognate PPRE from the promoter of the rat mHMG-CoAS gene (Rodriguez *et al.*, 1994). As shown in Figure 2A, cotransfection with an expression vector expressing PPAR α led to 2- and 4-fold increases in reporter gene activity over basal levels in the absence or presence of the PPAR α activator Wy-14,643, respectively. This is in agreement with previous reports using a reporter gene that contained this PPRE (Rodriguez *et al.*, 1994). Interestingly, coexpression of mHMG-CoAS led to a dramatic and dose-dependent increase in PPAR α -mediated transactivation, resulting in 200- to 300-fold inductions over basal levels of the reporter gene alone. This mHMG-CoA-mediated stimulation was dependent on the presence of PPAR α and a PPRE-containing reporter gene. Stimulation was specific to mHMG-CoAS, as cHMG-CoAS had no effect on PPAR α activity (Figure 2A). Significantly, the L393V mutant was unable to potentiate PPAR α activity. Indeed, L393V actually served as a transdominant inhibitor, resulting in the abrogation of activation by PPAR α .

To determine if the effects of mHMG-CoAS on PPAR α activity are general, we tested a reporter gene that contained the PPRE from the rat acyl-CoA oxidase (AOX) gene (Tugwood *et al.*, 1992; Zhang *et al.*, 1993). In this case, an expression vector for RXR α was included in the transfections, since endogenous levels of RXR α in these cells are insufficient for strong activation from this PPRE (Marcus *et al.*, 1993). As shown in Figure 2B, PPAR α /

RXR α strongly induced expression of this reporter gene in the absence or presence of ligand (7- and 28-fold, respectively), as expected. However, coexpression of either mHMG-CoAS or the L393V derivative antagonized PPAR-mediated activation. No effect was observed with cHMG-CoAS. Our findings indicate that mHMG-CoAS stimulates PPAR α activity in a PPRE-dependent manner, and demonstrate that the integrity of the LXXLL motif is necessary for this to occur.

PPAR α redirects mHMG-CoAS to the nucleus in transiently transfected cells

For mHMG-CoAS to play a role in PPAR α function, one would expect it to be present in the nucleus. Subcellular localization studies of human mHMG-CoAS have not been reported; however, the rat mHMG-CoAS has been localized by immunocytochemistry predominantly to mitochondria (Royo *et al.*, 1995; Serra *et al.*, 1996), although the presence of small amounts of mHMG-CoAS in other subcellular compartments has not been excluded. We examined the subcellular localization of mHMG-CoAS by attaching a hemagglutinin epitope tag (HA) to the C-terminus of the protein and carrying out immunofluorescent studies of transiently transfected cells. Addition of an HA tag to the C-terminus did not compromise activity, as mHMG-CoA-HA retained its ability to stimulate PPAR α -mediated transactivation *in vivo* (Figure 3B) and to interact with PPAR α *in vitro* (data not shown). Both the wild-type and mutant proteins were expressed *in vivo* and had electrophoretic mobilities consistent with that predicted for the mature 53 kDa protein, as determined by immunoblot analysis (Figure 3C). A less abundant species with an electrophoretic mobility corresponding to the predicted precursor form (57 kDa) was also present in each case (Figure 3C). Immunofluorescence of both mHMG-CoAS-HA and L393V-HA gave a punctate pattern of staining, consistent with mitochondrial localization (Figure 3A, panels 3 and 5, respectively). Nuclear staining over background was not evident, in agreement with previous findings on rat mHMG-CoAS (Serra *et al.*, 1996). However, when PPAR α was coexpressed, the cellular distribution of mHMG-CoAS was dramatically altered. In this case, prominent nuclear staining of mHMG-CoAS-HA was observed, in addition to punctate staining (Figure 3A, panel 4; ~80% of transfected cells exhibited nuclear staining). In contrast, coexpression of PPAR α did not alter the staining pattern of L393V-HA (Figure 3A, panel 6). Our findings suggest that, under certain conditions, PPAR α is able to redirect mHMG-CoAS to the nucleus and, moreover, that this requires the integrity of the receptor-interacting LXXLL motif.

Discussion

Our findings reveal a novel and unexpected pathway of gene regulation in which the product of a PPAR α target gene, predominantly found in mitochondria where it functions as a key ketogenic enzyme, can also specifically stimulate its own nuclear transcription by serving as a coregulator of PPAR α . Evidence exists for similar autoregulatory mechanisms with other nuclear hormone receptors. For example, it has been shown recently that the product of the rat *hairless* gene, whose transcription

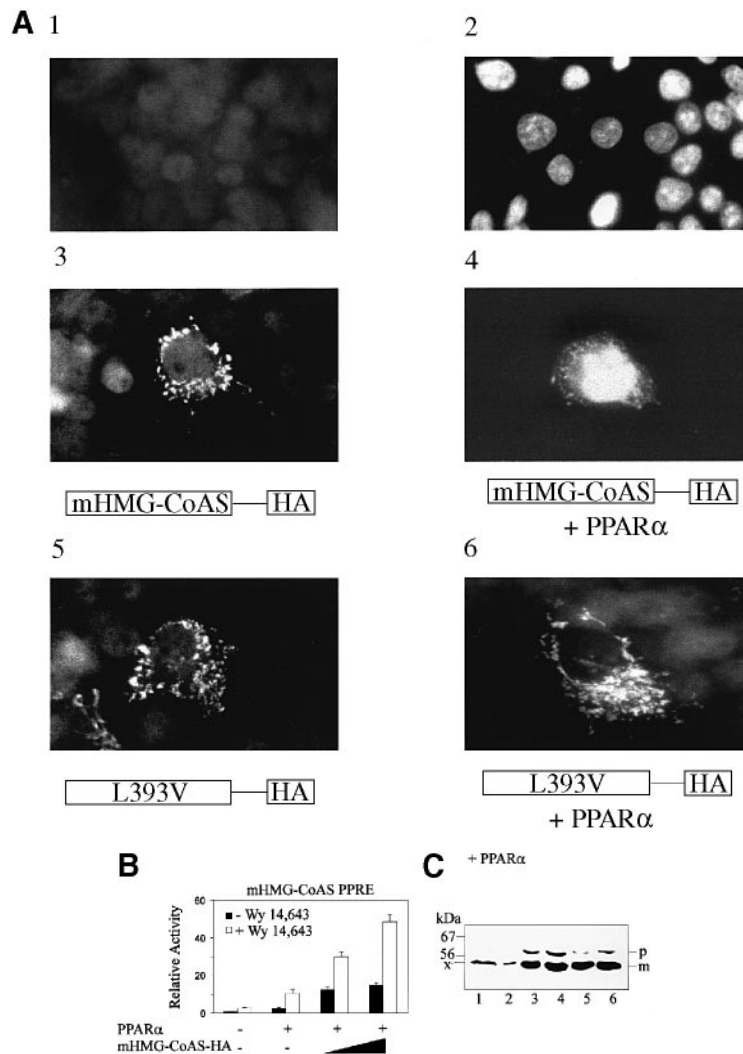


Fig. 3. mHMG-CoAS localizes to the nucleus in the presence of coexpressed PPAR α . **(A)** Panels show immunofluorescence using anti-HA antibodies of cells transfected with mHMG-CoAS or L393V containing an HA epitope tag at the C-terminus (mHMG-CoAS-HA and L393V-HA, respectively) alone, or in the presence of a PPAR α expression vector, as indicated. Also shown are untransfected cells (panel 1) and cells stained with Hoechst 33258 to visualize nuclei (panel 2). **(B)** Luciferase activity of the mHMG-CoAS-PPRE reporter gene construct transfected with expression plasmids for PPAR α alone, or with mHMG-CoAS-HA, as indicated. Cells were transfected as in Figure 2. Values are averages (\pm SEM) from two separate transfections and were normalized to the reporter gene alone, which is set as 1. **(C)** Cell extracts prepared from cells transfected with mHMG-CoAS-HA or L393V-HA alone (lanes 3 and 5, respectively), or in the presence of a PPAR α expression vector (lanes 4 and 6, respectively), were analyzed by immunoblotting with anti-HA antibodies. Lanes 1 and 2 are extracts prepared from untransfected cells, or cells transfected with empty expression vector, respectively. The molecular mass of mature mHMG-CoA-HA (m) is predicted to be 53 kDa, whereas the precursor (p) form is predicted to be ~57 kDa. (x) indicates a non-specific cross-reacting protein present in Cos-1 cells that migrates slightly more slowly than the predicted mature form of mHMG-CoAS-HA. The position of molecular mass markers is indicated.

is induced by the thyroid hormone receptor, interacts with this receptor and acts as a transcriptional repressor, thereby modulating expression of its own gene (Thompson and Bottcher, 1997).

Evidence presented herein indicates that mHMG-CoAS shares a number of properties with well-characterized nuclear hormone receptor coactivators (Glass *et al.*, 1997; Shibata *et al.*, 1997). First, mHMG-CoAS binds to PPAR α *in vitro* and stimulates its activity *in vivo*. Secondly, mHMG-CoAS contains a nuclear receptor-interacting consensus motif (LXXLL) that is found commonly in many nuclear hormone receptor-interacting cofactors. The integrity of this motif is required for efficient binding to PPAR α and stimulation of transactivation. Thirdly, a derivative of mHMG-CoAS, in which this motif is mutated, functions as a transdominant inhibitor of PPAR α -mediated trans-

activation. Fourthly, mHMG-CoAS accumulates in the nucleus in the presence of coexpressed PPAR α . Taken together, these findings suggest that mHMG-CoAS is a bona fide coregulatory factor for PPAR α .

The mechanism by which mHMG-CoA stimulates PPAR α activity is not known at present. We have been unable to detect a higher order protein-DNA complex containing PPAR α /RXR α /mHMG-CoAS using mobility shift analysis with the mHMG-CoA-PPRE and *in vitro* synthesized proteins (data not shown). However, this does not preclude the possibility of such a complex forming *in vivo*, where additional factors or events may be required for its formation and/or stability. The observation that the L393V derivative acts as a transdominant inhibitor of PPAR α transactivation is consistent with the possibility that mHMG-CoAS interacts both with PPAR α and with

a limiting downstream effector target(s) of PPAR α . Similar findings were reported with the receptor coactivator SRC-1, in which mutation of LXXLL motifs resulted in dominant-negative effects, a result attributed to sequestration of downstream effector molecules such as p300/CBP (Heery *et al.*, 1997). Therefore, mHMG-CoAS may function through the recruitment of other coregulatory factors required for PPAR α function. Nuclear hormone receptor cofactors function cooperatively in multimeric transcription complexes, and the recruitment, assembly, composition and function of cofactor complexes are differentially dependent upon the nature of the receptor, the response element and the availability of ligand (Kamei *et al.*, 1996; Chen *et al.*, 1997; DiRenzo *et al.*, 1997; Korzus *et al.*, 1998; Kurokawa *et al.*, 1998). It is interesting to note in this context that mHMG-CoAS-mediated stimulation was observed with the mHMG-CoAS-PPRE but not with the AOx-PPRE, indicating that the nature of the PPRE plays a determining role in the recruitment and/or function of mHMG-CoAS in cells. For instance, structural differences in the PPREs may dictate conformational changes in bound PPAR-RXR heterodimers, which in turn preclude or promote access of cofactors. Indeed, corepressors NCoR and SMRT can access PPAR α in solution but not when it is bound on the AOx-PPRE (DiRenzo *et al.*, 1997). Finally, it is interesting to note that mHMG-CoAS is itself acetylated and acts as an acetyltransferase in catalyzing the synthesis of HMG-CoA. Recent findings have established the importance of the acetylation of histones and general transcription factors in nuclear receptor-mediated gene regulation, and many nuclear receptor coactivators contain intrinsic or associated protein acetyltransferase activity (Ogryzko *et al.*, 1996; Chen *et al.*, 1997; Imhof *et al.*, 1997). We currently are investigating if the enzymatic activity and/or acetylation of mHMG-CoAS is required for the observed stimulation of PPAR α transactivation *in vivo*.

The observation that mHMG-CoAS accumulates in the nucleus in the presence of PPAR α supports a nuclear function for this protein. There are a growing number of examples of proteins that function in more than one subcellular compartment, and various mechanisms have been proposed to explain the differential targeting of proteins to different locations in the cell (Danpure, 1995). The mechanism by which a protein that contains a mitochondrial targeting sequence penetrates the nucleus in the presence of PPAR α is unclear at present but appears to involve a direct association between the two proteins, since the subcellular distribution of the L393V mutant derivative was unaltered in the presence of PPAR α . Nuclear translocation of mHMG-CoAS does not, however, appear to be due to PPAR-dependent differences in processing of the putative mHMG-CoAS mitochondrial targeting signal, since we observed no significant differences in the abundance and size of the steady-state levels of the processed and mature forms of mHMG-CoAS expressed in transiently transfected cells in the presence or absence of PPAR α (Figure 3C). Our data at present do not allow us to distinguish whether the precursor or mature form of mHMG-CoAS is responsible for nuclear stimulation of PPAR transactivation. We are currently addressing this issue since it bears directly on the pathway by which mHMG-CoAS gains access to the nucleus. Nuclear hor-

mone receptor-mediated translocation of receptor-binding proteins from one subcellular compartment to the nucleus is not without precedent. Rat deoxyuridine triphosphatase, which binds to PPAR α and antagonizes its activity, is shuttled from the cytosol to the nucleus by PPAR α (Chu *et al.*, 1996). Moreover, calreticulin, a calcium-binding protein that resides almost exclusively in the endoplasmic reticulum, has been shown to bind to, and inhibit the transcriptional activity of, several steroid and nuclear hormone receptors (Burns *et al.*, 1994). Consistent with our findings with mHMG-CoAS, it recently has been demonstrated that the glucocorticoid receptor enhances the nuclear accumulation of calreticulin (Roderick *et al.*, 1997).

In summary, we have shown that a mitochondrial protein that participates in an essential metabolic pathway can also be targeted to the nucleus where it functions in transcription, alluding to a potentially important pathway of nuclear-mitochondrial dialogue. This dual capacity suggests that mHMG-CoAS plays a multifunctional role in the cell; however, the physiological significance of the interaction between mHMG-CoAS and PPAR α remains to be elucidated. Ketogenesis is crucial for the generation of lipid-derived energy during periods of carbohydrate deprivation and in diseases such as diabetes, and this pathway is subject to multiple levels of regulation, including the rate of delivery and β -oxidation of fatty acids in the liver, and the production and utilization of acetyl-CoA (McGarry and Foster, 1980). The simplest interpretation of our findings is that mHMG-CoAS is involved in a positive feedback loop that serves to amplify its own expression in response to various metabolic and physiological conditions that demand increased ketone body formation. The observation that mHMG-CoAS may also influence the expression of other PPAR-responsive genes, such as acyl-CoA oxidase, the rate-limiting enzyme in the peroxisomal β -oxidation pathway, suggests that mHMG-CoAS may play an integrative role along with PPAR α in the maintenance and regulation of metabolic homeostasis.

Materials and methods

Plasmids

Plasmids were manipulated according to standard procedures (Ausubel *et al.*, 1997). We previously isolated a partial cDNA clone of human mHMG-CoAS, encoding amino acids 1–345 with respect to the precursor protein, in a two-hybrid screen for PPAR α -interacting factors (Miyata *et al.*, 1996). A full-length, 2 kbp cDNA encoding the complete 508 amino acid human mHMG-CoAS, which includes the putative 37 amino acid mitochondrial targeting sequence (Mascaró *et al.*, 1995), was constructed using a previously isolated 3' fragment of human mHMG-CoAS cDNA (Boukafane *et al.*, 1994) and cloned into the *in vivo* and *in vitro* expression plasmid pSG5 (Promega). The full-length cDNA clone for hamster cHMG-CoAS (Gil *et al.*, 1986) was also cloned into pSG5. pGST-RXR α and pGST-PPAR α express the respective full-length receptors as GST fusion proteins and were cloned in the vectors pGEX-2T and pGEX-2TK (Pharmacia), respectively. *In vivo* and *in vitro* expression vectors for mPPAR α , human RXR α and the luciferase reporter gene construct pAOx(X2)*luc*, which contains two copies of the rat AOx-PPRE, have been described (Zhang *et al.*, 1992). pHMG*luc* is a luciferase reporter gene construct containing a single copy of the rat mHMG-CoAS-PPRE, and was constructed by cloning a synthetic double-stranded oligonucleotide (upper strand: 5'-GATCCTGACTTGTCTGAGACCTTTGGCCAGTTTTTCTGAGGCAGGCAGAGGA-3') corresponding to the published PPRE sequence (Rodriguez *et al.*, 1994) into the *Bam*HI site of pCPS*luc* (Zhang *et al.*, 1992). The L393V mutant of mHMG-CoA, cloned in pSG5, was created by site-directed

mutagenesis using the oligonucleotide 5'-GGTGCCTCGGCTCGGTT-CTGTCCACC-3' (altered base underlined) and a commercially available kit (Stratagene). Versions of the wild-type mHMG-CoAS and the L393V mutant containing a single in-frame copy of the HA epitope tag at their C-termini were constructed in the mammalian expression vector pcDNA3 (Invitrogen). Briefly, a *Sac*II site was generated by site-directed mutagenesis immediately upstream of the natural termination codon in the mHMG-CoAS cDNA, into which a double-stranded synthetic oligonucleotide encoding the HA epitope was inserted.

Protein-binding assays

GST pull-down assays were carried out as previously described (Miyata *et al.*, 1996) using GST-PPAR α or GST-RXR α immobilized to glutathione-Sepharose (Pharmacia) and [³⁵S]methionine-labeled mHMG-CoAS, cHMG-CoAS or mutant L393V proteins synthesized *in vitro* by transcription-translation. Bound proteins were analyzed by SDS-PAGE on 10% gels and quantitated using a Molecular Dynamics Phosphor-Imager. Immunoprecipitations were carried out as described (Fujiki *et al.*, 1984). Briefly, radiolabeled mHMG-CoAS, cHMG-CoAS or mutant L393V proteins were incubated with equivalent amounts of unlabeled PPAR α or RXR α synthesized *in vitro* (normalization was determined in parallel experiments by [³⁵S]methionine incorporation) and incubated with polyclonal antiserum to mouse PPAR α or human RXR α , or with pre-immune serum, as appropriate (1:200 dilution). Complexes were recovered on protein A-conjugated agarose beads (Bio-Rad) and analyzed by SDS-PAGE.

Transfection assays

Transfection of BSC40 cells by the calcium phosphate method and measurement of luciferase activity were as described (Marcus *et al.*, 1993; Miyata *et al.*, 1993). Briefly, subconfluent BSC40 cells (2 \times 10⁵ cells/6 cm dish) were transfected with 1.25 μ g of reporter gene plasmid, 1.5 μ g of PPAR α and/or RXR α expression plasmid, and various amounts of the different HMG-CoAS expression plasmids, as described in the figure legends. Plasmid DNA was normalized to 5 μ g with sonicated salmon sperm DNA, and promoter dosage was kept constant by the addition of appropriate amounts of the corresponding empty vectors.

Immunofluorescence

Immunofluorescence was performed essentially as described (Ausubel *et al.*, 1997). Cos-1 cells, grown on coverslips, were transfected with plasmids expressing HA-tagged proteins (0.5 μ g), as above. Cells were fixed at room temperature with 4% paraformaldehyde in phosphate-buffered saline for 10 min, followed by blocking with 2% goat serum/0.2% Triton X-100 for 30 min. Permeabilized cells were incubated with mouse monoclonal anti-HA antibody (12CA5, Boehringer Mannheim, 1:15 dilution) for 1 h at 37°C and stained with Texas red-conjugated goat anti-mouse secondary antibody (Jackson Immunoresearch Labs, 1:30 dilution). Nuclei were stained by treating cells at -70°C in methanol for 10 min, followed by incubation at 37°C for 4 h with 0.25 mg of Hoechst 33258/ml.

Immunoblot analysis

Immunoblot analysis was carried out using standard procedures (Ausubel *et al.*, 1997). Briefly, extracts from Cos-1 cells, transfected as above but using 1.25 μ g of the HA-tagged expression plasmids, were prepared using RIPA buffer [10% NP-40, 0.4% deoxycholate, 66 mM EDTA, 10 mM Tris-HCl (pH 7.4), 1 mM phenylmethylsulfonyl fluoride (PMSF)]. Proteins (75 μ g) were resolved by SDS-PAGE on 10% gels, transferred to Hybond nitrocellulose membranes (Amersham), and incubated with mouse anti-HA antibody (1:500 dilution), followed by horseradish peroxidase-conjugated sheep anti-mouse IgG (1:1000 dilution). Proteins were detected by chemiluminescence using a commercially available kit (Amersham).

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References

- Ausubel,F., Brent,R., Kingston,R.E., Moore,D.D., Seidman,J.G., Smith,J.A. and Struhl,K. (1994) *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc., New York, NY.
- Ayté,J., Gil-Gómez,G., Haro,D., Marrero,P.F. and Hegardt,F.G. (1990) Rat mitochondrial and cytosolic 3-hydroxy-3-methylglutaryl CoA synthases are encoded by two different genes. *Proc. Natl Acad. Sci. USA*, **87**, 3874-3878.
- Braissant,O., Foufelle,F., Scotto,C., Dauca,M. and Wahli,W. (1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR- α , - β and - γ in the adult rat. *Endocrinology*, **137**, 354-366.
- Boukafane,Y., Duncan,A., Wang,S., Labuda,D., Robert,M.-F., Sarrazin,J., Schappert,K. and Mitchel,G.A. (1994) Human mitochondrial HMG CoA synthase: liver cDNA and partial genomic cloning, chromosome mapping to 1p12-p13 and possible role in vertebrate evolution. *Genomics*, **23**, 552-559.
- Burns,K., Duggan,B., Atkinson,E.A., Famuiliski,K.S., Nemwer,M., Bleackley,R.C. and Michalak,M. (1994) Modulation of gene expression by calcitriol binding to the glucocorticoid receptor. *Nature*, **367**, 476-480.
- Chen,H.W., Lin,R.J., Schiltz,R.L., Chakravarti,D., Nash,A., Nagy,L., Privalsky,M.L., Nakatani,Y. and Evans,R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*, **90**, 569-580.
- Chu,R., Lin,Y., Rao,S. and Reddy,J.K. (1996) Cloning and identification of rat deoxyuridine triphosphatase as an inhibitor of peroxisome proliferator-activated receptor α . *J. Biol. Chem.*, **271**, 27670-27676.
- Danpure,C.J. (1995) How can the product of a single gene be localized to more than one intracellular compartment? *Trends Cell Biol.*, **5**, 230-238.
- Devchand,P.R., Keller,H., Peters,J.M., Vazquez,M., Gonzalez,F.J. and Wahli,W. (1996) The PPAR α -leukotriene B₄ pathway to inflammation control. *Nature*, **384**, 39-43.
- DiRenzo,J., Söderström,M., Kurokawa,R., Ogliastro,M.H., Ricote,M., Ingrey,S., Horlein,A., Rosenfeld,M.G. and Glass,C.K. (1997) Peroxisome proliferator-activated receptors and retinoic acid receptors differentially control the interactions of retinoid X receptor heterodimers with ligands, coactivators and corepressors. *Mol. Cell Biol.*, **17**, 2166-2176.
- Fujiki,Y., Rachubinski,R.A. and Lazarow,P.B. (1984) Synthesis of a major integral membrane polypeptide of rat liver peroxisomes on free polysomes. *Proc. Natl Acad. Sci. USA*, **81**, 7127-7131.
- Gil,G., Goldstein,J.L., Slaughter,C.A. and Brown,M.S. (1986) Cytoplasmic 3-hydroxy-3-methylglutaryl coenzyme A synthase from the hamster. I. Isolation and sequencing of a full-length cDNA. *J. Biol. Chem.*, **261**, 3710-3716.
- Glass,C.K., Rose,D.W. and Rosenfeld,M.G. (1997) Nuclear receptor coactivators. *Curr. Opin. Cell Biol.*, **9**, 222-232.
- Heery,D.M., Kalkhoven,E., Hoare,S. and Parker,M.G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature*, **387**, 733-736.
- Imhof,A., Yang,X.J., Ogryzko,V.V., Nakatani,Y., Wolffe,A.P. and Ge,H. (1997) Acetylation of general transcription factors by histone acetyltransferases. *Curr. Biol.*, **7**, 689-692.
- Jiang,C.Y., Ting,A.T. and Seed,B. (1998) PPAR- γ agonists inhibit production of monocyte inflammatory cytokines. *Nature*, **391**, 82-86.
- Kamei,Y. *et al.* (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell*, **85**, 403-414.
- Kliwer,S.A., Umesono,K., Noonan,D.J., Heyman,R.A. and Evans,R.M. (1992) Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. *Nature*, **358**, 771-774.
- Korzus,E., Torchia,J., Rose,D.W., Xu,L., Kurokawa,R., McInerney,E.M., Mullen,T.M., Glass,C.K. and Rosenfeld,M.G. (1998) Transcription factor-specific requirements for coactivators and their acetyltransferase functions. *Science*, **279**, 703-707.
- Kurokawa,R., Kalafus,D., Ogliastro,M.H., Kioussi,C., Xu,L., Torchia,J., Rosenfeld,M.G. and Glass,C.K. (1998) Differential use of CREB binding protein coactivator complexes. *Science*, **279**, 700-703.
- Latruffe,N. and Vamecq,J. (1997) Peroxisome proliferators and peroxisome proliferator activated receptors (PPARs) as regulators of lipid metabolism. *Biochimie*, **79**, 81-94.

- Lemberger,T., Desvergne,B. and Wahli,W. (1996) Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. *Annu. Rev. Cell Dev. Biol.*, **12**, 335–363.
- Marcus,S.L., Miyata,K.S., Zhang,B., Subramani,S., Rachubinski,R.A. and Capone,J.P. (1993) Diverse peroxisome proliferator-activated receptors bind to the peroxisome proliferator-responsive elements of the rat hydratase/dehydrogenase and fatty acyl-CoA oxidase genes but differentially induce expression. *Proc. Natl Acad. Sci. USA*, **90**, 5723–5727.
- Mascaró,C., Buesa,C., Ortiz,J., Haro,D. and Hegardt,F.G. (1995) Molecular cloning and tissue expression of human mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase. *Arch. Biochem. Biophys.*, **317**, 385–390.
- McGarry,J.D. and Foster,D.W. (1980) Regulation of hepatic fatty acid oxidation and ketone body production. *Annu. Rev. Biochem.*, **49**, 395–420.
- Miyata,K.S., Zhang,B., Marcus,S.L., Capone,J.P. and Rachubinski,R.A. (1993) Chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds to a peroxisome proliferator-responsive element and antagonizes peroxisome proliferator-mediated signaling. *J. Biol. Chem.*, **268**, 19169–19172.
- Miyata,K.S., McCaw,S.E., Patel,H.V., Rachubinski,R.A. and Capone,J.P. (1996) The orphan nuclear hormone receptor LXR α interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. *J. Biol. Chem.*, **271**, 9189–9192.
- Nagy,L., Tontonoz,P., Alvarez,J.G.A., Chen,H.W. and Evans,R.M. (1998) Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR γ . *Cell*, **93**, 229–240.
- Ogryzko,V.V., Shiltz,R.L., Russanova,V., Howard,B.H. and Nakatani,Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell*, **87**, 953–959.
- Puigserver,P., Wu,Z.D., Park,C.W., Graves,R., Wright,M. and Spiegelman,B.M. (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*, **92**, 829–839.
- Quant,P.A. (1994) The role of mitochondrial HMG-CoA synthase in regulation of ketogenesis. *Essays Biochem.*, **28**, 13–25.
- Roderick,H.L., Campbell,A.K. and Llewellyn,D.H. (1997) Nuclear localisation of calreticulin *in vivo* is enhanced by its interaction with glucocorticoid receptors. *FEBS Lett.*, **405**, 181–185.
- Rodriguez,J.C., Gil-Gomez,G., Hegardt,F.G. and Haro,D. (1994) Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. *J. Biol. Chem.*, **269**, 18767–18772.
- Royo,T., Pedragosa,M.J., Ayte,J., Gil-Gomez,G., Vilaro,S. and Hegardt,F.G. (1995) Immunolocalization of mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase in rat liver. *J. Cell Physiol.*, **162**, 103–109.
- Schoonjans,K., Staels,B. and Auwerx,J. (1996) The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim. Biophys. Acta*, **1302**, 93–109.
- Serra,D., Bellido,D., Asins,G., Arias,G., Vilaró,S. and Hegardt,F.G. (1996) The expression of mitochondrial 3-hydroxy-3-methylglutaryl-coenzyme-A synthase in neonatal rat intestine and liver is under transcriptional control. *Eur. J. Biochem.*, **237**, 16–24.
- Shibata,H., Spencer,T.E., Onate,S.A., Jensterm G., Tsai,S.Y., Tsai,M.-J. and O'Malley,B.W. (1997) Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. *Recent Prog. Horm. Res.*, **52**, 141–164.
- Thompson,C.C. and Bottcher,M. (1997) The product of a thyroid hormone-responsive gene interacts with thyroid hormone receptors. *Proc. Natl Acad. Sci. USA*, **94**, 8527–8532.
- Tugwood,J.D., Issemann,I. anderson,R.G., Bundell,K.R., McPheat,W.L. and Green,S. (1992) The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *EMBO J.*, **11**, 433–439.
- Voegel,J.J., Heine,M.J.S., Tini,M., Vivat,V., Chambon,P. and Gronemeyer,H. (1998) The coactivator TIF2 contains three nuclear receptor-binding motifs and mediates transactivation through CBP binding-dependent and -independent pathways. *EMBO J.*, **17**, 507–519.
- Zhang,B., Marcus,S.L., Sajjadi,F.G., Alvares,K., Reddy,J.K., Subramani,S., Rachubinski,R.A. and Capone,J.P. (1992) Identification of a peroxisome proliferator-responsive element upstream of the gene encoding rat peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase. *Proc. Natl Acad. Sci. USA*, **89**, 7541–7545.
- Zhang,B., Marcus,S.L., Miyata,K.S., Subramani,S., Capone,J.P. and Rachubinski,R.A. (1993) Characterization of protein–DNA interactions within the peroxisome proliferator-responsive element of the rat hydratase-dehydrogenase gene. *J. Biol. Chem.*, **268**, 12939–12945.

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