# Peroxisomal bifunctional enzyme binds and activates the activation function-1 region of the peroxisome proliferator-activated receptor α

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The transcriptional activity of peroxisome proliferator-activated receptors (PPARs), and of nuclear hormone receptors in general, is subject to modulation by cofactors. However, most currently known co-activating proteins interact in a ligand-dependent manner with the C-terminal ligand-regulated activation function (AF)-2 domain of nuclear receptors. Since PPARα exhibits a strong constitutive transactivating function contained within an N-terminal AF-1 region, it can be speculated that a different set of cofactors might interact with this region of PPARs. An affinity purification approach was used to identify the peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (bifunctional enzyme, BFE) as a protein which strongly and specifically interacted with the N-terminal 92 amino acids of PPARα. Protein–protein interaction assays with the cloned BFE confirmed this interaction, which could be mapped to amino acids 307–514 of the BFE and the N-terminal 70 amino acids of PPARα. Moreover, transient transfection experiments in hepatoma cells revealed a 2.2-fold increase in the basal and ligand-stimulated transcriptional activity of PPARα in the presence of BFE. This stimulatory effect is preferentially observed for the PPARα isoform and it is significantly stronger (4.8-fold) in non-hepatic cells, which presumably express lower levels of endogenous BFE. Hence, the BFE represents the first known cofactor capable of activating the AF-1 domain of PPAR without requiring additional regions of this receptor. These data are compatible with a model whereby the PPAR-regulated BFE is able to modulate its own expression through an enhancement of the activity of PPARα, representing a novel peroxisomal–nuclear feed-forward regulatory loop.

Key words: co-activator, nuclear hormone receptor, peroxisomal β-oxidation, transcriptional activation.

## INTRODUCTION

The activation of transcription by nuclear hormone receptors is modulated by cofactors enhancing or silencing their transcriptional activity [1]. A variety of proteins with co-activator or co-repressor function have hitherto been identified, and some have been demonstrated to be functionally relevant *in vivo* [2–6]. However, most currently identified co-activators directly interact with the C-terminal portion of the ligand-binding domain (LBD) of nuclear receptors, which contains a ligand-dependent activation function (AF) motif AF-2 [7–9]. Similarly, most of the known co-activators for the peroxisome proliferator-activated receptors (PPARs) interact in a ligand-enhanced manner with the conserved C-terminal helix 12 (AF-2 region), as demonstrated by protein–protein interaction studies and X-ray crystallography [7,10]. However, most nuclear receptors, including PPARs, also demonstrate a constitutive transcriptional response, which is particularly pronounced for PPARα, whose basal activity in certain cells is significantly higher than the augmentation observed by the addition of exogenous ligand [11]. We have previously shown that this basal transactivation resides within the N-terminal 70 amino acids of PPARα, thereby defining a ligand-independent activating function AF-1. Therefore we have now attempted to identify nuclear proteins interacting constitutively with the AF-1 region of PPARα. Using an affinity-purification approach, we were able to identify the peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (bifunctional enzyme, BFE) as a strong interaction partner and activator of PPARα, raising the possibility of a peroxisomal–nuclear signalling loop.

## EXPERIMENTAL

### Construction of plasmids

pSG5-human (h) PPARα, pSG5-hRXRα, the DR1- and UAS-luciferase reporters, the fusion constructs of GAL4BD-hPPARα (amino acids 1–92; GAL4BD is yeast transcription activator DNA-binding domain), obtained by cloning the cDNA corresponding to amino acids 1–92 of PPAR downstream of the yeast GAL4 DNA-binding domain (DBD) (amino acids 1–147), and the fusion protein GST–ERα (glutathione S-transferase fused to oestrogen receptor α; amino acids 80–150), as well as the plasmids for the rat BFE and the peroxisomal trihydroxycoprostanoyl-CoA oxidase (THCOX) were described previously [12–14]. The cDNAs for the two peroxisomal enzymes were subcloned into the T/A pCR3.1 expression vector (Invitrogen BV, Groningen, The Netherlands). GST–hPPARα (amino acids 1–92) fusion protein was obtained by cloning the cDNA corresponding to amino acids 1–92 into the *Bam*HI/*Eco*RI sites of pGEX-2T (Amersham Pharmacia Biotech).

### Protein overexpression and purification

GST or GST fusion proteins were expressed in DH5α bacteria according to the manufacturer's (Amersham Pharmacia Biotech) protocol. The supernatant of the bacterial lysate was coupled to

Abbreviations used: (h)PPAR, (human) peroxisome proliferator-activated receptor; RXR, retinoid X receptor; BFE, bifunctional enzyme (peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase); THCOX, peroxisomal trihydroxycoprostanoyl-CoA oxidase; PGC, PPARγ co-activator; AF, activation function; MAPK, mitogen-activated protein kinase; ETYA, 5,8,11,14-eicosatetraynoic acid; LBD, ligand-binding domain; DBD, DNA-binding domain; GST, glutathione S-transferase; (h)ERα, (human) oestrogen receptor α; cPGI, carbaprostacyclin; GAL4BD, yeast transcription activator DNA-binding domain.

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a GST–Sephadex resin (Amersham Pharmacia Biotech) by incubating for 1 h, followed by three washing steps, and then kept in PBS as a slurry of 50 %. Phosphorylation of the fusion proteins was performed as follows: 5 $\mu$l of the 50 % GST–Sephadex slurry in 50 mM Tris, pH 7.5, was incubated with mitogen-activated protein kinase (MAPK) buffer, 100 $\mu$M ATP and 50 units of p42 MAPK (New England Biolabs via Bio-Concept, Allschwil, Switzerland) for 30 min at 30 °C, followed by washing three times with PBS. *In vitro* transcription and translation of proteins was performed with the TnT T7 Quick kit (Promega via Catalys AG, Wallisellen, Switzerland) in the presence of [$^{35}$S]methionine.

### Preparation of nuclear extracts and subcellular fractionation

Nuclear extracts were prepared as described elsewhere [15]. Other subcellular fractions were prepared as described in [16].

### Determination of catalase activity

Catalase activity was assayed spectrophotometrically by measuring the kinetics of the decrease in absorption of $H_2O_2$ at 230 nm during 90 s ($A_1$ at $t = 0$ s, $A_2$ at $t = 90$ s) after the addition of the sample, as previously described [17]. Catalase activity was then determined by calculating the first-order rate constant ($k$, units $s^{-1}$) according to the following equation:

$$k = (2.3/\Delta t) \cdot \log (A_1/A_2)$$

where $\Delta t$ is 90 s [17].

### GST pull-down experiments

A 5 $\mu$l portion of 50 % GST slurry in binding buffer (20 mM Hepes, pH 7.7, 75 mM KCl, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 0.05 % Nonidet P40, 10 % (v/v) glycerol and 2 mM freshly added DTT) were incubated at room temperature for 1 h with 5 $\mu$l of *in vitro*-translated and $^{35}$S-labelled proteins and 10 $\mu$l of binding buffer. For nuclear extract, 20 $\mu$g of protein were incubated with 10 $\mu$l of 50 % GST slurry overnight at 4 °C. The incubation mixture was centrifuged and the pellet washed three times with binding buffer. SDS/PAGE denaturing sample buffer was added to the final pellet and the samples were run on an SDS/10 %-(w/v)-PAGE gel. Gels were autoradiographed or silver-stained with the Silver Stain Kit (Bio-Rad Laboratories AG, Glattbrugg, Switzerland).

### Protein preparation and identification

Preparative incubations with 200 $\mu$l of 50 % GST slurry and 200 $\mu$g of nuclear extract were performed for subsequent protein analysis. Proteins were separated by SDS/PAGE and stained with Coomassie Blue. The bands of interest were cut, and processed for mass spectroscopy on matrix-assisted laser-desorption ionization (MALDI) target plates (Perseptive Biosystems Voyager Elite MALDI–time-of-flight MS) [18]. Protein identification was carried out using SmartIdent (http://www.expasy.ch/sprot/SmartIdent.html), which allows the identification of proteins using pI, molecular mass and peptide mass fingerprinting data. The experimentally measured peptide masses were compared with the theoretical peptides calculated for all proteins in the SWISS-PROT/TREMBL databases. Subsequently, the band of interest was further characterized by MS/MS sequencing using a tandem mass spectrometer (Q-TOF) from MicroMass (Manchester, U.K.) [19]. Fragment ion spectra were interpreted with the SEQUEST database search (Finnigan).

### Cell culture and transfection

HepG2 and NIH 3T3 cells were grown and transfected by the calcium phosphate methods as described in [11,20]. At 18 h after transfection, the cells were treated with vehicle, or 1 $\mu$M of 5,8,11,14-eicosatetraynoic acid (ETYA), carbaprostacyclin (cPGI; Cayman Chemical, Ann Arbor, MI, U.S.A.) or BRL49653 (SmithKline Beecham, Harlow, Essex, U.K.) as appropriate. After 42 h the cells were harvested and luciferase activity determined with the LucLite™ kit (Packard, Zurich, Switzerland) using a LumiCount™ instrument (Packard). Luciferase activity was normalized by protein content, rather than for an internal standard, which subject to promoter squelching with the reporter plasmid used [21,22]. Four independent experiments were performed in triplicate, except for the data represented in Figure 4(B) (below), which are derived from three independent experiments (perfomed in triplicate). Data are expressed as means ± 1 S.E.M. Statistical differences were assessed by the non-parametric Mann–Whitney $U$-test. A significant difference was defined as a $P < 0.05$.

## RESULTS

### The N-terminus of PPARα specifically interacts with two peroxisomal enzymes present in liver nuclear extracts

Using the GST–PPARα (amino acids 1–92) fusion protein coupled to a GSH column, we identified two 68–75 kDa proteins in rat liver nuclear extracts which specifically interacted with PPARα, but not the GST moiety alone or the AF-1 region of the ER (GST–ERα amino acids 80–150) (Figures 1 and 2A). The two protein bands were excised and characterized by protease footprinting and MS sequencing. The higher-mobility band was identified as the BFE (75 kDa), whereas THCOX (68 kDa) constituted the lower-mobility band. The presence of two peroxisomal enzymes in nuclear extracts raised the possibility of a contamination of our nuclear preparations by the peroxisomal fraction. However, when catalase activity is used as a peroxisomal marker in the various cellular fractions, we found less than 3 % of the catalase activity in the nuclear fractions compared with the peroxisomes (Table 1). Hence we have no evidence for a major contamination of the nuclear extract by peroxisomal proteins.

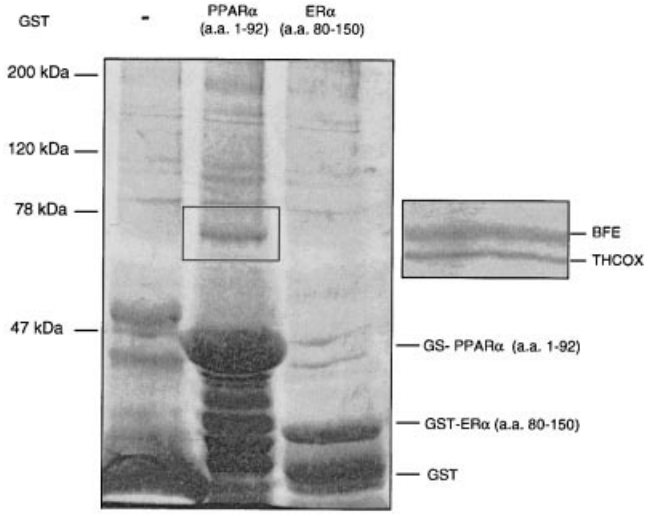


**Figure 1 Interaction of the N-terminus of PPARα with nuclear proteins**

Affinity chromatography with 10 $\mu$l of either GST (lane 1), GST–hPPARα (amino acids 1–92) (lane 2) or GST–hERα (amino acids 80–150) (lane 3) coupled to a solid phase. A 20 $\mu$g portion of rat liver nuclear extract was incubated overnight at 4 °C. After washing, the eluted samples were subjected to SDS/PAGE and the gel was silver-stained. Two bands of apparent molecular masses 68 and 75 kDa binding specifically to GST–hPPARα (amino acids 1–92) were identified.

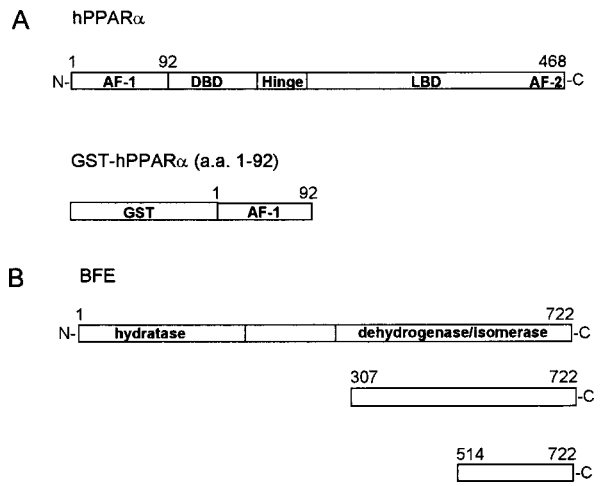


**Figure 2 Functional domains of PPARα and BFE**

(**A**) PPARα contains the activation-function AF-1 (amino acids 1–92), the DBD and hinge region, as well as the C-terminal LBD; (**B**) The BFE is composed of an N-terminal moiety harbouring the hydratase activity, while the C-terminus contains the dehydrogenase/isomerase function.

## BFE, but not THCOX, specifically interacts with the N-terminus of PPARα

In order to verify the presence of a direct physical interaction between these peroxisomal enzymes and PPARα, we performed GST pull-down experiments with *in-vitro*-translated BFE and THCOX. Figure 3(A) illustrates the strong binding of BFE (60–90% of input) to amino acids 1–92 of PPARα. However, THCOX neither interacted with the N-terminus of PPARα, nor did it alter its interaction with BFE. Since we have previously shown that the phosphorylation of the AF-1 region of PPARα by MAPK enhances its transcriptional activity, we *in vitro* phosphorylated the N-termini of PPARα and ERα with p42 MAPK [12]. However, this phosphorylation did not modify the interaction of PPARα with BFE (Figure 3B). The phosphorylated AF-1 region of ERα was used as a negative control. Finally, we created two N-terminal truncations of BFE, both of which eliminated the enzymically active sites for the hydratase activity of the BFE (Figure 2B) located within amino acids 1–280. While the BFE fragment containing amino acids 307–722 still interacted strongly with PPARα, the further deletion of amino acids 307–514 completely abolished this interaction, although the *in vitro* expression of the amino acids 514–722 fragment of the BFE was unaltered (Figure 3C). Deletion of the C-terminus (amino acids 426–722) abolished the interaction (results not shown), suggesting that region of amino acids 307–426 is necessary for binding.

## BFE enhances the basal and ligand-dependent transcriptional activity of PPARα

In order to examine whether the BFE was able to modulate the transcriptional activity of PPARα in liver cells, we performed transient transfection experiments in the HepG2 hepatoma cell line. The cells were transfected with PPARα and retinoid receptor Xα (RXRα), as well as a DR1-luciferase reporter with different quantities of the expression vector for BFE and its truncated mutants. As shown in Figure 4(A), the wild-type BFE stimulated in HepG2 cells the basal activity of PPARα equally well as its ligand (ETYA) in the absence of BFE ($P < 0.001$). Moreover,

**Table 1 Catalase activity in liver cellular fractions**

Catalase activity is expressed as *k*/mg of protein for each cellular fraction. $k = (2.3/\Delta t) \cdot \log(A_1/A_2)$, where $\Delta t$ is 90 s. The relative catalase activity expresses the fraction of catalase activity with respect to the peroxisomes. SN represents the final supernatant of the last centrifugation for the obtention of heavy peroxisomes (see the Experimental section for details).

| Cellular fraction | Catalase activity (*k*/mg of protein) | Relative catalase activity |
|---|---|---|
| Nuclear extract | 5.8 | 0.03 |
| Nuclei | 4.4 | 0.02 |
| Mitochondria | 5.3 | 0.03 |
| SN | 16.8 | 0.09 |
| Peroxisomes | 198 | 1.00 |

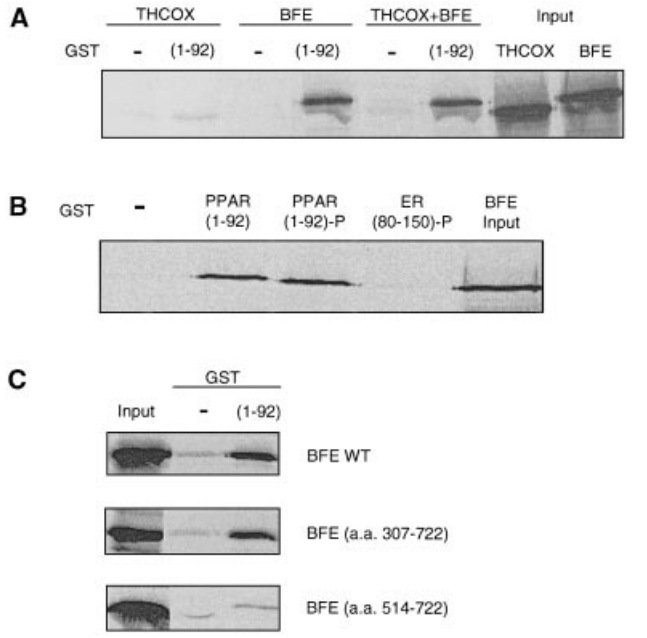


**Figure 3 Interaction of the N-terminus of PPARα with *in-vitro*-translated BFE and THCOX and mapping of the interaction domain**

(**A**) A 5 μl portion of 50% slurry of either GST or GST–hPPARα [amino acids (a.a.) 1–92] were incubated with 5 μl of $^{35}$S-labelled *in-vitro*-translated THCOX (lanes 1 and 2) or BFE (lanes 3 and 4), or both (lanes 5 and 6). Lanes 7 and 8 represent the input quantities of $^{35}$S-labelled *in-vitro*-translated THCOX and BFE respectively. (**B**) A 5 μl portion of 50% slurry of either GST (lane 1), GST–hPPARα (amino acids 1–92) (lane 2), MAPK-phosphorylated GST–hPPARα (amino acids 1–92) (lanes 3), or phosphorylated GST–ERα (amino acids 80–150) (lane 4) were incubated with 5 μl of $^{35}$S-labelled *in-vitro*-translated BFE (input in lane 5). (**C**) For mapping of the interaction region within the BFE, GST pull-down experiments were performed as described above, using either GST (lane 1), or GST–hPPARα (amino acids 1–92), together with 5 μl of $^{35}$S-labelled *in-vitro*-translated wild-type (WT) BFE or one of two truncated variants (BFE amino acids 307–722 and amino acids 514–722).

the effect of the BFE was additive to the activation by the ligand ($P < 0.001$). However, neither the truncated variants of the BFE, nor THCOX, significantly modulated transactivation by the full-length PPAR. Similarly, the BFE only minimally altered the transcriptional activity of a GAL4BD fusion protein with the ligand-binding domain of PPARα (results not shown). Since the HepG2 hepatoma cells are likely to express significant amounts of endogenous BFE, we performed similar experiments in the NIH3T3 fibroblast cell line (Figure 4B). In these cells the stimulatory effect of BFE on the basal activity of PPARα was stronger compared with that observed in HepG2 cells ($P < 0.001$). In addition, these experiments revealed that the

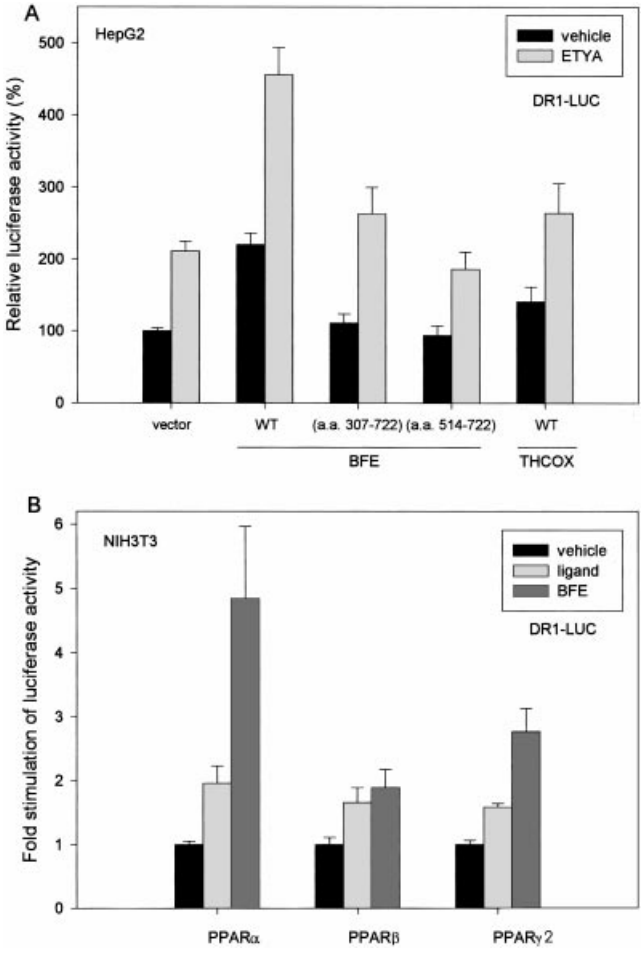


**Figure 4 BFE enhances the transcriptional activity of full-length PPARα**

(**A**) HepG2 cells were transiently transfected with 0.2 μg of pSG5-hPPARα, 0.2 μg of pSG5-hRXRα, 1 μg of DR1-luciferase reporter gene and 1.4 μg of pCR3.1 vector, wild-type (WT) pCR3.1-BFE, pCR3.1-BFE (amino acids 307–722), pCR3.1-BFE (amino acids 514–722) or pCR3.1-THCOX, as indicated. At 18 h after transfection, cells were treated with 1 μM ETYA or vehicle and harvested 24 h later for the determination of luciferase activity, which was normalized for protein content and expressed relative to the activity of hPPARα in the presence of pCR3.1 empty vector and vehicle. The full-length BFE (WT) significantly activated PPARα in the presence and absence of ligand ($P < 0.001$), whereas the truncated variants and the THCOX had no significant effect. (**B**) NIH3T3 cells were transfected as in (**A**) with pSG5-hPPARα, pCDNA-hPPARβ or pSG5-hPPARγ2 and then stimulated with 1 μM ETYA, cPGI or BRL49653 respectively. The BFE significantly stimulated the basal activity of PPARα, PPARβ or PPARγ2 ($P < 0.001$, $P < 0.05$, and $P < 0.001$ respectively). Abbreviation: a.a., amino acid(s).

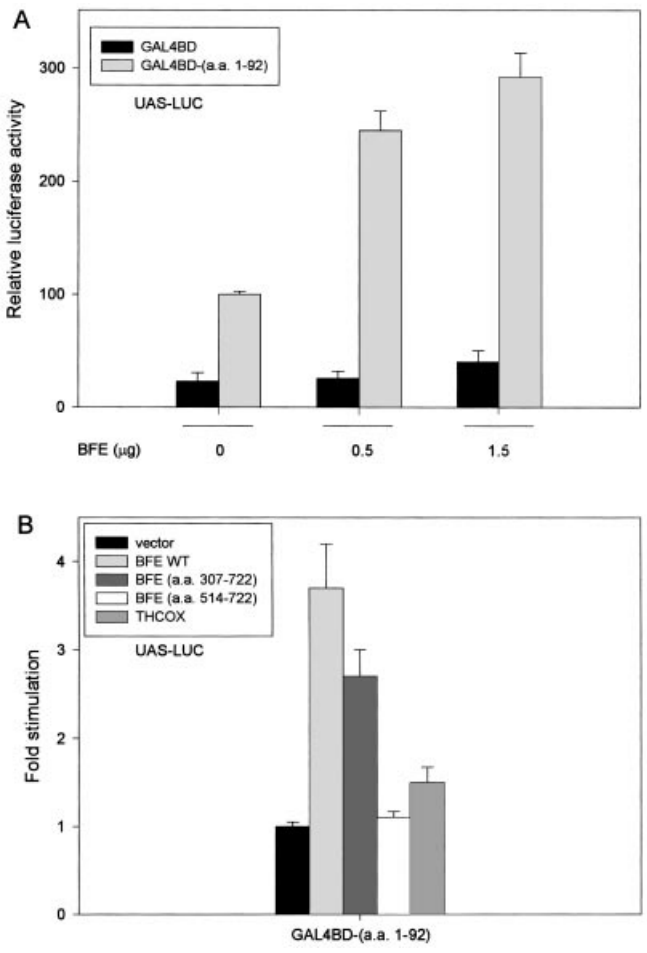


**Figure 5 Activation of the isolated N-terminus of PPARα by BFE, and functional mapping of the activating region of BFE**

(**A**) HepG2 cells were transfected with 1 μg of GAL4BD or GAL4BD-hPPARα [amino acids (a.a.) 1–92] together with 0.4 μg of the UAS-luciferase reporter gene and various amounts of pCR3.1-wild-type BFE as indicated. Cells were harvested 42 h after transfection and luciferase activity was determined. The results were normalized for protein concentrations. At all quantities tested, the BFE significantly activated the GAL4BD-hPPARα (amino acids 1–92) ($P < 0.001$), whereas no significant effect was observed on the GAL4BD. (**B**) The transfections were identical with those of (**A**), except that either 3 μg of pCR3.1 vector, wild-type pCR3.1-BFE (WT), pCR3.1-BFE (amino acids 307–722), pCR3.1-BFE (amino acids 514–722) or pCR3.1-THCOX were co-transfected. A strong and significant activation was obtained with the BFE WT and BFE (amino acids 307–722) ($P < 0.001$), whereas the BFE (amino acids 514–722) had no significant, and THCOX only a quantitatively marginal ($P < 0.05$), effect on GAL4BD (amino acids 1–92).

BFE also activated the PPARβ and PPARγ isoforms ($P < 0.05$), although to a lesser degree than PPARα.

### The AF-1 region of PPARα is sufficient for the activation by BFE

Since we have initially identified the BFE as a protein interacting with the AF-1 region of PPARα, we then examined whether amino acids 1–92 of PPAR were sufficient to mediate the transcriptional response. Figure 5(A) illustrates the more-than-2-fold enhancement of the constitutive transcriptional activity of the GAL4BD–PPARα (amino acids 1–92) fusion protein ($P < 0.001$), with no significant effect on the activity of the GAL4BD moiety. When the activating potential of the various fragments of the BFE was examined, their capability to interact with PPAR (Figure 3C) correlated well with their transactivating capacity ($P < 0.001$ and $< 0.05$ for the BFE WT and amino acids 307–522 respectively), with the BFE amino acids 514–722 being devoid of any activating potential (Figure 5B). However, while the BFE amino acids 307–722 strongly interacted and activated the N-terminus of PPARα, this fragment of the BFE did not activate the full-length PPARα (Figure 3).

### Amino acids 1–70 of PPARα are sufficient to mediate the activation by BFE

Our previous data suggested that amino acids 1–70 of PPARα are sufficient for the constitutive AF-1 activity [12]. Similarly, the data shown in Figure 6 demonstrate that the same region of PPARα is necessary and sufficient to mediate activation by the BFE, suggesting that this peroxisomal cofactor is a *bona fide* co-activator of the AF-1 region (2.5-fold stimulation for amino

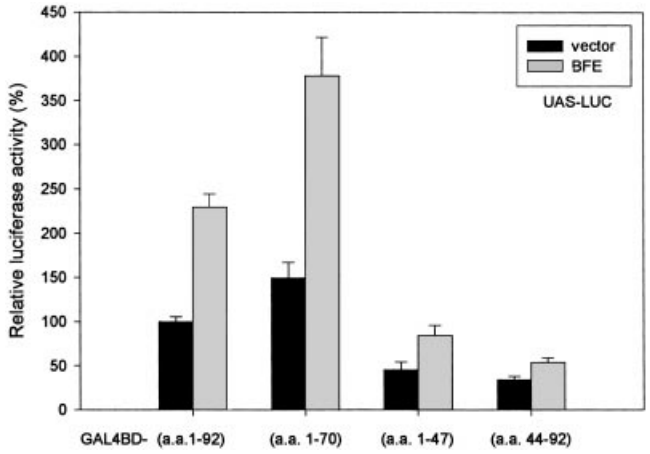


**Figure 6 Functional mapping of the activating region within the N-terminus of PPARα**

HepG2 cells were co-transfected with 1 μg of GAL4BD or its fusion constructs together with various fragments of the N-terminus of hPPARα (amino acids 1–92, 1–70, 1–44 and 44–92) in the presence of 0.4 μg of the UAS-luciferase reporter and 1.5 μg of either pCR3.1 vector or pCR3.1-BFE. Cells were harvested 42 h after transfection, and luciferase activity was determined. The results were normalized for protein concentrations and expressed as percentages of the basal activity of GAL4BD-hPPARα (amino acids 1–92). BFE strongly activates the amino acids 1–92 and 1–70 fragments of PPARα (2.3- and 2.5-fold respectively; $P < 0.001$), whereas the fragments containing amino acids 1–47 and 44–92 were activated to a lesser extent (1.9- and 1.6-fold respectively; $P < 0.05$).

acids 1–70, $P < 0.001$). In contrast, the smaller fragments (amino acids 1–47 and 44–92) were less well activated by the BFE (1.9- and 1.6-fold respectively; $P < 0.05$).

## DISCUSSION

Although a variety of co-activators interacting with the ligand-activated C-terminal AF-2 region of nuclear receptors have been described, little is known about the cofactors mediating the ligand-independent basal transcriptional activity observed for some receptors. We now describe the identification of the peroxisomal BFE as an activator of the AF-1 domain of PPARα with which it interacts strongly in a constitutive manner. This stimulatory effect is preferentially observed for the PPARα isoform and it is significantly stronger in a fibroblast cell line, which is presumed to express lower levels of endogenous BFE.

The observation that a metabolic enzyme is able to modulate the transcriptional activity of a nuclear receptor is intriguing, but not without precedent. Recently, a mitochondrial ketogenic enzyme which is regulated itself by PPARs was shown to interact with and activate the transcriptional activity of PPARα through a LXXLL motif, thereby amplifying its own expression [23]. Similarly, the BFE represents a well known target gene for PPARs, which contains functional PPAR response elements in its promoter [24]. Hence, a pattern emerges whereby pivotal mitochondrial or peroxisomal enzymes regulated by PPARs have an additional function as regulators of the activity of PPARα, thereby providing a positive feed-forward regulation. Such regulatory loops between cytoplasmic and nuclear compartments may represent a novel mechanism for adjusting the expression levels of rate-limiting metabolic enzymes to metabolic needs.

The mechanism by which the BFE augments the basal transcriptional activity of PPARα is currently speculative. However, it is clear that the region of the enzyme containing the active sites for the hydratase activity is dispensable, while the N-terminal interaction interface of PPARα corresponds to amino acids 1–70. We have previously shown this very same region to be sufficient for the basal activity of PPARα, thereby delimiting the AF-1 domain. The interaction between the AF-1 region and the BFE does not appear to involve the classical LXXLL motif, as no such sequence can be localized within the BFE. It can be hypothesized that the BFE may modulate the recruitment of proteins such as histone acetylases or other transactivating proteins or, alternatively, that it may modify the intracellular

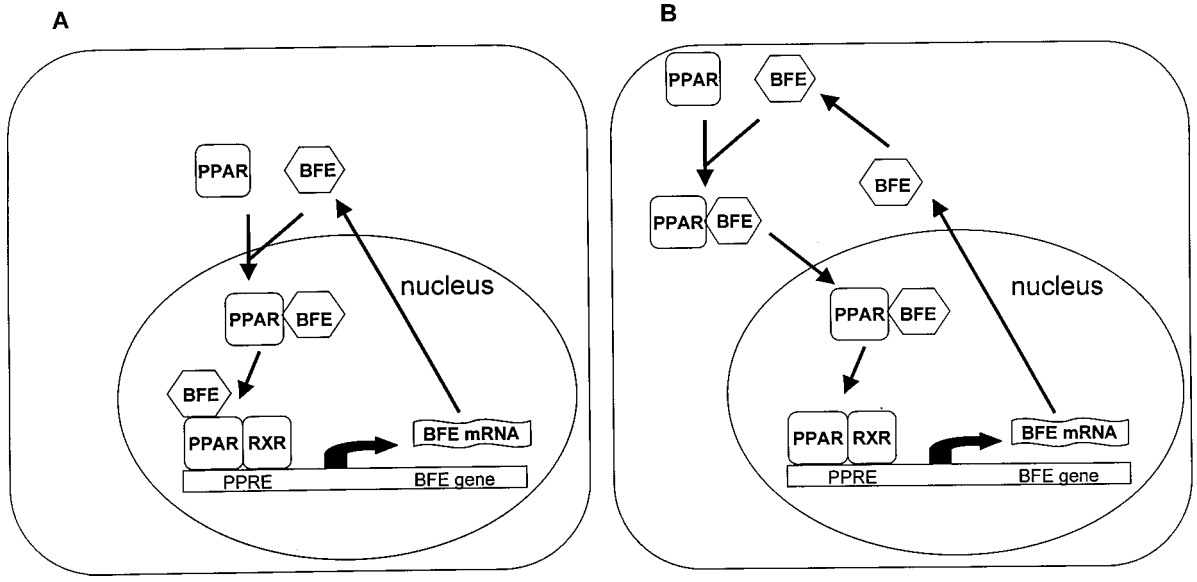


**Figure 7 Possible mechanisms of activation of PPARα by the BFE**

PPARα is an inducer of the expression of the peroxisomal BFE through a PPAR-response element (PPRE) located in the regulatory region of the BFE gene [24]. The BFE in turn is able to interact physically with the N-terminus of PPAR, thereby enhancing its transcriptional activity through an as yet unknown mechanism. It can be speculated that the BFE acts either as a transcriptional cofactor at the nuclear level (**A**) or, alternatively, that the BFE modulates the intracellular trafficking (e.g. nuclear import) of PPARα (**B**).

trafficking of PPARα, e.g. its import into the nucleus (Figure 7). While virtually all presently known co-activators for PPARs interact with their hinge or ligand-binding domains, the PPARγ co-activator-2 (PGC-2) represents another AF-1-specific co-activator besides BFE [25]. However, although the N-terminal 138 amino acids of PPARγ represent the major binding site for PGC-2, additional, more distal regions of PPAR are required for transactivation. Hence, the BFE appears to be the first co-factor which is capable of binding to, and activating, the isolated N-terminal AF-1 domain of PPAR. Although the C-terminal moiety of the BFE is sufficient for activating the AF-1 domain of PPARα, the entire BFE molecule is required to enhance transactivation by the full-length PPARα. This observation may suggest that the activating domain of the BFE resides within amino acids 307–722, but that its activity is blocked by non-AF-1 domain(s) of the full-length PPARα, unless the entire BFE is present.

Finally, we have also shown that a second peroxisomal enzyme, THCOX catalysing the first step of the peroxisomal oxidation of the CoA esters of bile acid intermediates, also interacts with the N-terminus of PPARα. However, this interaction is only detectable when hepatic nuclear extracts are used, whereas isolated THCOX does not interact with, and only poorly activates, PPARα. These findings suggest that THCOX and BFE might physically interact, which can be speculated to result in a ternary complex between PPAR, BFE and THCOX *in vivo*. However, our experiments could not substantiate the formation of such a complex *in vitro*. Alternatively, it is possible that only endogenous, but not *in-vitro*-translated, THCOX protein is capable of interacting with PPARα, raising the possibility that THCOX might be subject to post-translational modifications.

In summary, our data demonstrate that the peroxisomal BFE has an AF-1 specific transactivating function, suggesting a novel model whereby the PPAR-regulated BFE is able to modulate the activity of PPARα through a novel peroxisomal–nuclear feed-forward loop.

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