Activity and subcellular compartmentalization of peroxisome proliferator-activated receptor α are altered by the centrosome-associated protein CAP350

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Summary

Peroxisome proliferator-activated nuclear hormone receptors (PPAR) are ligand-activated transcription factors that play pivotal roles in governing metabolic homeostasis and cell growth. PPARs are primarily in the nucleus but, under certain circumstances, can be found in the cytoplasm. We show here that PPARα interacts with the centrosome-associated protein CAP350. CAP350 also interacts with PPARδ, PPARγ and liver-X-receptor α, but not with the 9-cis retinoic acid receptor, RXRα. Immunofluorescence analysis indicated that PPARα is diffusely distributed in the nucleus and excluded from the cytoplasm. However, in the presence of coexpressed CAP350, PPARα colocalizes with CAP350 to discrete nuclear foci and to the centrosome, perinuclear region and intermediate filaments. In contrast, the subcellular distribution of RXRα or of thyroid hormone receptor α was not altered by coexpression of CAP350. An amino-terminal fragment of CAP350 was localized exclusively to nuclear foci and was sufficient to recruit PPARα to these sites. Mutation of the single putative nuclear hormone receptor interacting signature motif LXXLL present in this fragment had no effect on its subnuclear localization but abrogated recruitment of PPARα to nuclear foci. Surprisingly, mutation of the LXXLL motif in this CAP350 subfragment did not prevent its binding to PPARα in vitro, suggesting that this motif serves some function other than PPARα binding in recruiting PPARα to nuclear spots. CAP350 inhibited PPARα-mediated transactivation in an LXXLL-dependent manner, suggesting that CAP350 represses PPARα function. Our findings implicate CAP350 in a dynamic process that recruits PPARα to discrete nuclear and cytoplasmic compartments and suggest that altered intracellular compartmentalization represents a regulatory process that modulates PPAR function.

Supplementary material available online at
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Introduction

Peroxisome proliferator-activated receptors (PPAR) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. The three major PPAR isoforms [α, δ (also called β) and γ] have distinct tissue expression patterns and ligand sensitivities and regulate the expression of complex, interactive gene networks that govern energy and metabolic homeostasis, glucose utilization, cell growth and differentiation (Berger and Moller, 2002; Bocher et al., 2002; Hihi et al., 2002; Michalik and Wahli, 2002; Lee et al., 2003). Dysregulation of PPAR function has been implicated directly in the etiology and pathogenesis of diabetes, obesity, hyperlipidemia, atherosclerosis, cancer, inflammation and other disorders (Kersten et al., 2000; Vosper et al., 2002; Lee et al., 2003).

PPARs activate or repress gene expression in response to cognate ligands by binding in conjunction with the obligate heterodimerization partner, retinoid-X-receptor (RXR), to specific cis-acting regulatory elements called peroxisome proliferator-response elements (PPRE) present in the promoter regions of target genes (Berger and Moller, 2002; Shearer and Hoekstra, 2003). PPAR function is controlled at multiple levels, including receptor abundance and stability, ligand availability, protein-protein interaction and posttranslational modification. Activity is manifested through both ligand-dependent and ligand-independent recruitment of numerous receptor-interacting coregulatory molecules into multicomponent transcription complexes. Coregulatory complexes include coactivators or corepressors and contain a number of associated or intrinsic enzymatic activities, such as histone acetylase and deacetylase, that remodel chromatin and/or modify various components of the transcriptional machinery (Xu et al., 1999; Qi et al., 2002).

An additional important regulatory mechanism for nuclear hormone receptors involves processes that regulate their intracellular trafficking and subcellular compartmentalization. The importance of dynamic, ligand-dependent subcellular distribution is well established for members of the steroid hormone receptor subfamily such as the glucocorticoid and progesterone receptors (reviewed by Baumann et al., 1999).

In the absence of cognate hormone, steroid hormone receptors, referred to as type I receptors, are normally present in the cytoplasm in large multicomponent complexes that contain heat shock proteins and chaperones. Ligand binding induces conformational rearrangements that allow translocation of steroid hormone receptors into the nucleus, where they bind to target gene response elements as homodimers. In contrast, the so-called type II nuclear hormone receptors, which include PPARs, RXR, vitamin D receptor, thyroid hormone receptor, retinoic acid receptor and numerous orphan receptors, are thought to reside constitutively in the nucleus irrespective of the presence of ligand. However, recent studies using green fluorescent protein (GFP) chimeras have indicated that some type II nuclear hormone receptors, including the receptors for thyroid hormone, vitamin D, retinoic acid and estrogen, can shuttle between the nucleus and cytoplasm in a dynamic, energy-dependent manner (Baumann et al., 1999; Hager et al., 2002; Maruvada et al., 2003). In some cases, receptors have been shown to accumulate in distinct nuclear foci in the presence of ligand (van Steensel et al., 1995). Intracellular trafficking and subcellular or subnuclear compartmentalization may be a general phenomenon of type II nuclear hormone receptors and could be differentially influenced by ligand interaction, association with cofactors and/or through heterodimerization with RXR (Baumann et al., 2001b; Mackem et al., 2001; Akiyama et al., 2002; Barsony and Prufer, 2002; Prufer and Barsony, 2002; Prufer et al., 2002).

PPARs are predominately nuclear, and their cellular distribution is apparently unaffected by exogenous ligand, their phosphorylation status or their interaction with corepressors or coactivators such as NCoR, SMRT or SRC-1 that are known to modulate PPAR transcriptional activity (Aikiyama et al., 2002; Berger et al., 2002). However, there is evidence for an altered subcellular distribution of PPAR isoforms under certain circumstances. PPARα is present constitutively in the cytoplasm of differentiated human macrophages, whereas PPARγ is present in the nuclei of these cells (Chinetti et al., 1998). All three PPAR isoforms are cytoplasmic in the endothelial cell line EVC-304 and translocate into the nucleus in the presence of the PPARγ activator, 15-deoxy-Δ^{12-14} prostaglandin J2 (Bishop-Bailey and Hla, 1999). Interestingly, RXR has been shown to alter the nuclear distribution of PPARγ from a diffuse to a reticulated pattern but has no such effect on the distribution of PPARα or PPARδ (Akiyama et al., 2002). Lastly, recent studies indicate that a subpopulation of PPARα may be present in rat liver cytosol in complex with the molecular chaperone, hsp90 (Sumanasekera et al., 2003).

The mechanisms that modulate differential PPAR subcellular distribution and any potential correlation of PPAR subcellular distribution with PPAR function are unknown. It is probable that these processes are mediated by protein-protein interactions; however, with the exception of the selective effects of RXRα on PPARγ nuclear redistribution described above, the existence of cellular factors that may influence PPAR subcellular localization has not been reported. We show here that PPARα functionally and physically interacts with CAP350, a putative centrosome-associated protein of unknown function. We demonstrate that CAP350 is concentrated in the nucleus in discrete foci, or inclusions, and in the cytoplasm as part of the centrosome and in association with intermediate filaments (IF). In the presence of CAP350, PPARα, which is diffuse in the nucleus, redistributes and colocalizes with CAP350 in subnuclear foci, and associates with the perinuclear region, the centrosome and microfilaments in the cytoplasm. Transfection assays using a PPAR-responsive reporter gene demonstrate that CAP350 inhibits PPARα-mediated transactivation. Our findings implicate CAP350 in a dynamic process that mediates distinct pathways of subcellular compartmentalization of PPARα and which may be of consequence in regulating the function of this nuclear receptor within the cell.

Materials and Methods

Cells
NIH3T3 cells (ATCC CRL 1658) were maintained as monolayers in Dulbecco's minimum essential medium containing 10% fetal bovine serum, 1% penicillin/streptomycin and 1% glutamine. Rat hepatoma H4IIEC3 cells (ATCC CRL 1600) were maintained as monolayers in Dulbecco's minimum essential medium containing 10% horse serum, 5% fetal bovine serum, 1% penicillin/streptomycin and 1% glutamine.

Plasmids
pGAD-KM3 contains a 1.9 kb insert of a partial cDNA encoding human CAP350 (GenBank NM_014810.2; NP_055625) previously isolated from a HeLa cell activation domain library by two-hybrid screening using mouse PPARα as bait (Miyata et al., 1996). pGST-KM3 was constructed by isolating the *Sma*I-*Xho*I fragment from pGAD-KM3 and inserting it into the *Sma*I site of pGEX-2TK (Amersham). Reporter gene plasmids pTK-*Luc*, pTK-PPRE($\times$3)-*Luc* and pCMVLacZ, cloning vector pSG5, and mammalian in vitro and in vivo expression vectors for human PPARα, PPARγ$_2$, PPARδ, RXRα and LXRα and rat thyroid hormone receptor α (TRα) have been described previously (Willy et al., 1995; Miyata et al., 1996; Kassam et al., 1998; Berger et al., 2002; Hunter et al., 2001). Fluorescent protein expression plasmids pECFP-Mito, pEYFP-α-tubulin and pECFP-Golgi were obtained from Clontech. pGFP-NUP98 was a kind gift from Maureen Powers, Emory University. pGFP-SF2/ASF was a kind gift from David Spector, Cold Spring Harbor Laboratory. pGFP-PML was a kind gift from Tetsutaro Sata, National Institute of Infectious Diseases, Tokyo, Japan. pMT-hCAP350 is a mammalian expression vector encoding the full-length cDNA for human CAP350 tagged with the c-Myc epitope and was a kind gift from Ludger Klein-Hitpass, Clinical University, Essen, Germany. The plasmid pEYFP-CAP350$_{1-890}$, which expresses amino acids 1-890 of CAP350 fused to the EYFP fluorophore, was generated by PCR amplification of CAP350 cDNA and insertion into the vector pEYFP-C1 (Clontech). pEYFP-CAP350$_{1-890(LSHAA)}$ expresses EYFP fused to the amino-terminal 890-amino acid fragment of CAP350 containing alanine substitutions of leucine residues at positions 762 and 763 and was generated from pEYFP-CAP350$_{1-890}$ by site-directed mutagenesis using the QuikChange Mutagenesis System (Stratagene) according to the manufacturer's instructions. pGST-CAP350$_{1-890}$ and pGST-CAP350$_{1-890(LSHAA)}$ contain the above 1-890-residue wild-type and mutant fragment derivatives, respectively, cloned into pGEX2-TK. pmRFP-PPARα expresses full-length PPARα fused to monomeric red fluorescent protein (mRFP) (Campbell et al., 2002) and was constructed by PCR amplification of the coding sequence of PPARα and insertion into mammalian expression vector pmRFP-C1. pmRFP-C1 was a kind gift from Joanna Graczyk (McMaster University) and was made by PCR amplification of mRFP cDNA and insertion into pEGFP-C1 (Clontech) to replace EGFP with mRFP cDNA.

Northern blot analysis

Northern blot analysis was done on the multiple tissue northern blots Human Blot MTN and Human Blot MTNII (Clontech) according to the manufacturer's protocol using as probe the *Bam*HI-*Kpn*I fragment of pGAD-KM3 labeled with $[\alpha\text{-}^{32}\text{P}]$dATP by random priming (Ausubel et al., 1994). Blots were probed with ^{32}P-labeled α-actin as an internal loading standard.

Protein binding assays

GST protein binding assays were carried out with receptor proteins labeled in vitro with $L\text{-}[^{35}\text{S}]$methionine using a coupled transcription/ translation system (Promega) and GST-KM3 fusion protein purified from *Escherichia coli* as previously described (Miyata et al., 1998). The GST-KM3 fusion protein was bound to glutathione-Sepharose 4B beads (Amersham) and incubated with labeled proteins. The beads were washed extensively and bound material was analyzed by SDS-PAGE. Where indicated, binding reactions were carried out in the presence of the hypolipidemic peroxisome proliferator and PPARα ligand Wy-14,643 (ChemSyn Laboratories, Harrisonville, MT, USA) at a final concentration of 100 μM, the RXRα ligand 9-cis retinoic acid (Sigma) at a final concentration of 10 μM, the LXRα ligand 22-[*R*]-hydroxycholesterol (Research Plus, Manasquan, NJ, USA) at a final concentration of 10 μM, or Panagonist B, an agonist that partially activates all three PPAR isoforms (Merck Pharmaceuticals, Rahway, NJ) at a final concentration of 3 μM.

Transient transfection assays and measurement of luciferase activity

Transient transfections of H4IIEC3 cells were carried out as described previously (Zhang et al., 1992; Marcus et al., 1993; Kassam et al., 1998) using Lipofectamine (Invitrogen). Briefly, cells (3×10^5 cells/ well in 6-well plates) were transfected with 4 μl of Lipofectamine together with 0.5 μg pTK-PPRE($\times$3)-*Luc*, various amounts of the CAP350 expression vector pMT-hCAP350 (0.1-0.5 μg/well), or the plasmids pEYFP-CAP350$_{1\text{-}890}$ or pEYFP-CAP350$_{1\text{-}890(\text{LSHAA})}$ expressing the wild-type or mutant form of an amino-terminal fragment of CAP350, respectively, and 0.1 μg of the β-galactosidase expression vector pCMVLacZ, which was used to control for transfection efficiency, as indicated in the legends to figures. Promoter dosage and plasmid concentration were kept constant by the addition of empty vector, as appropriate. Cells were incubated for 48 hours in medium minus phenol red and containing 10% charcoal-stripped fetal calf serum. Where indicated, Wy-14,643 was added to a final concentration of 100 μM from a stock solution prepared in DMSO. Control cells received equivalent amounts of vehicle. Cell lysates were prepared, and luciferase activity was determined. Reported values were normalized to protein content and β-galactosidase activity (Ausubel et al., 1994). NIH3T3 cells were transfected as above except that 0.1 μg each of PPARα and RXRα expression plasmids were included.

Immunofluorescence and imaging

Immunofluorescence staining was performed essentially as described previously (Ausubel et al., 1994). Briefly, NIH3T3 cells were grown on coverslips and transfected with 2 μg of plasmid and 6.6 μl of ExGen (MBI Fermentas, Burlington, Ontario, Canada) according to the manufacturer's protocol. At 24 hours posttransfection, cells were fixed at room temperature with 2% paraformaldehyde in PBS for 15 minutes, followed by permeabilization with 0.2% Triton X-100/2% calf serum for 10 minutes on ice. Permeabilized cells were incubated with the appropriate primary antibody (1:100-1:200 dilution) for 1 hour at 37°C, washed with PBS and incubated with the appropriate fluorescent secondary antibody (1:100 dilution). Nuclei were stained with Hoechst 33245 (Sigma) at 10 μg/ml during the first washing step after secondary antibody treatment. Where indicated, cells were treated with Brefeldin A (Sigma) at 1 mg/ml or actinomycin D (Sigma) at 1 mg/ml for 30 minutes at 37°C prior to fixation. Phalloidin-FITC (Sigma) was used according to the manufacturer's protocol to detect actin. Primary antibodies used included anti-human PPARα, anti-cytokeratin (AbCam, Cambridge, UK), anti-human c-Myc (BD Biosciences, Mississauga, Ontario, Canada), anti-pericentrin (Covance Research Products, Richmond, CA. USA), anti-rat TRα (Santa Cruz Biotechnology) and anti-human RXRα (a kind gift from Sandra Marcus, University of Alberta, Canada). Secondary antibodies included Texas Red-conjugated anti-mouse IgG (Jackson) and Alexa Fluor 488 anti-rabbit IgG (Molecular Probes).

All fluorescence microscope images were captured on a Nikon TE200 epifluorescence inverted microscope equipped with a 60$\times$ oil immersion plan apochromat objective and a Hamamatsu Orca 100 digital camera (Nikon). Z-stack images were captured using a Prior motorized stage and Simple PCI v5.2 imaging software (Compix, Cranberry Township, PA, USA), deconvolved using the nearest neighbors algorithm in Autodeblur v9.1 (Autoquant Imaging, Albany, NY, USA) and assembled into three-dimensional rotations using Imaris v4.0 (Bitplane Software, Zurich, Switzerland). Z-stacks were converted to voxels and further analyzed with the Imaris CoLoc module (Bitplane Software) to determine colocalization between channels in three dimensions after image thresholding. Two-dimensional images that were not quantitated were deconvolved using the 2D blind iterative deconvolution algorithm in Autodeblur v9.1. Fluorescence channels were captured sequentially using a Sutter Lambda 10-2 filter wheel system (Sutter Instruments, Novato, CA, USA) with Hoechst/DAPI/AMCA, EGFP and Texas Red filter sets (Chroma Technologies, Brattleboro, VT, USA).

For live cell microscopy, NIH3T3 cells were seeded onto heated culture dishes (Delta T; Bioptechs, Butler, PA, USA) and transfected with ExGen and 2 μg of plasmid expressing mRFP-PPARα, EYFP-CAP350, EYFP-CAP350$_{1\text{-}890}$, or EYFP-CAP350$_{1\text{-}890(\text{LSHAA})}$, as indicated. Cells were maintained at 37°C and observed 18-24 hours posttransfection directly in the culture dish and without fixation. Sequential images were digitally captured, and channels were overlaid and pseudocolored using Simple PCI v5.2 imaging software. The emission signal for mRFP was distinguished from that of EGFP or EYFP by combining the filter set for Texas Red with the filter set for EGFP or EYFP (Chroma Technologies).

Immunoblot analysis

Immunoblot analysis to detect the expression of CAP350 in transfected cells was carried out using standard procedures (Ausubel et al., 1994). Briefly, NIH3T3 cells were transfected with 1 or 2 μg of pMT-hCAP350, as described above. Cell extracts were prepared using RIPA buffer (10% NP-40, 0.4% deoxycholate, 66 mM EDTA, 10 mM Tris-HCl, pH 7.4, 1 mM PMSF). Proteins (25 μg) were resolved by SDS-PAGE, transferred to Hybond nitrocellulose membranes (Amersham) and incubated with mouse anti-c-Myc antibody (2 μg/ml), followed by HRP-conjugated sheep anti-mouse IgG (1:5000 dilution). Proteins were detected by chemiluminescence using a commercially available kit (Amersham), according to the manufacturer's instructions.

Results

CAP350 interacts with PPARα in vitro

We previously used PPARα as bait in a yeast two-hybrid screen to identify PPARα-interacting proteins from a HeLa cell cDNA activation domain library (Miyata et al., 1996). One cDNA clone, called KM3, was isolated from two independent screens and contained a 1.9 kb insert of a partial cDNA encoding an unknown protein that bound specifically to PPARα.

Fig. 1. Schematic of human CAP350. The CAP350 cDNA is predicted to encode a protein of 3117 amino acids. The locations of the conserved CAP-Gly domain and of the two putative LxxLL nuclear hormone receptor-interacting signature motifs in CAP350 are indicated. KM3 indicates the extent of two independently isolated, partial HeLa cell cDNA clones obtained from yeast two-hybrid screens for PPARα-interacting proteins (Miyata et al., 1996) and corresponds to amino acids 572-1176 of CAP350.
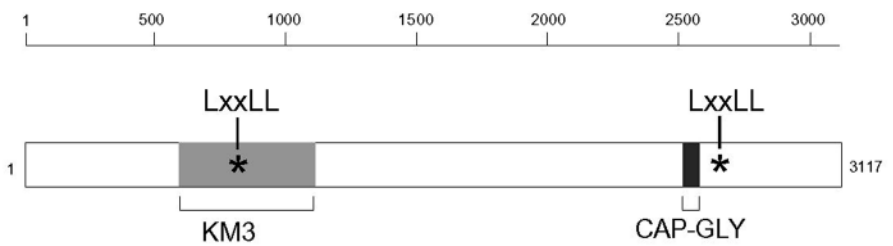
Comparison with genomic and cDNA sequences deposited in GenBank indicated that the KM3 clone encoded amino acids 572-1176 of CAP350, a putative human centrosome-associated protein (GenBank accession number NM_014810.2).

CAP350 is 3117 amino acids in length and of unknown function, but it is predicted to be associated with the centrosome and cytoskeleton by virtue of a CAP-Gly domain present near its carboxyl terminus (amino acids 2517-2559) (Fig. 1). CAP350 also contains two putative LxxLL signature motifs (amino acids 759-763, LSHLL, contained in the KM3 partial cDNA, and amino acids 2719-2723, LLDLL) that are known to be important in mediating protein-protein interactions with nuclear hormone receptors (Xu et al., 1999; Qi et al., 2002). The full-length CAP350 mRNA is predicted to be 11.9 kb in length and to encode a protein of approximately 351 kDa. Analysis of the 160 kb CAP350 gene (chromosomal location 1p36.13-q41) predicts that multiple mRNA species can be generated by alternative splicing, giving rise to several distinct proteins.

Northern blot analysis against human RNA from various tissues was performed to determine the tissue distribution pattern of CAP350 mRNA. CAP350 mRNA is most abundantly expressed in testis and in skeletal muscle, and to a lesser extent in several other tissues (Fig. 2). The size of the major RNA transcript (11.5 kb) is consistent with the predicted size of full-length CAP350 mRNA. PPARα is also expressed in skeletal muscle and testis (Shultz et al., 1999; Michalik

et al., 2002), suggestive of a physiologically relevant link between it and CAP350.

To determine if CAP350 and PPARα can interact with each other in vitro, we carried out glutathione S-transferase (GST) pull-down assays employing a bacterially synthesized GST-KM3 fusion protein and in vitro synthesized receptor proteins radiolabeled with L-[^{35}S]methionine. PPARα bound to GST-KM3 (approximately 8% of input radioactivity bound specifically to GST-KM3) but not to GST alone (Fig. 3A). Binding efficiency was not noticeably enhanced by the addition of the PPAR ligand, Panagonist B (Fig. 3A). As a control, we tested binding of GST-KM3 with an irrelevant radiolabeled protein, luciferase. As shown in Fig. 3A, luciferase
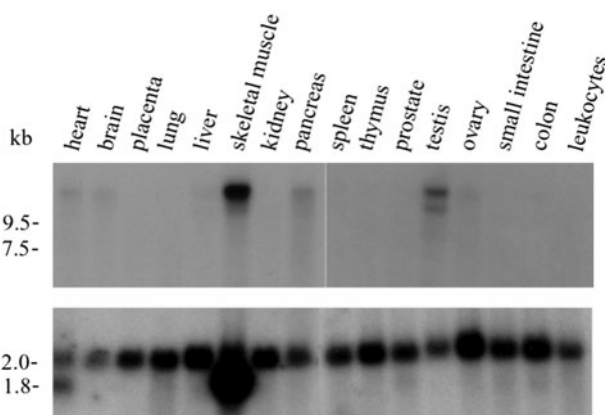

Fig. 2. Expression pattern of CAP350 mRNA. A northern blot of RNAs isolated from different human tissues was probed with a ^{32}P-labeled partial cDNA for CAP350. The same blot was probed with ^{32}P-labeled α-actin to serve as an internal standard for RNA loading. The positions of size markers, in kilobases (kb), are indicated.
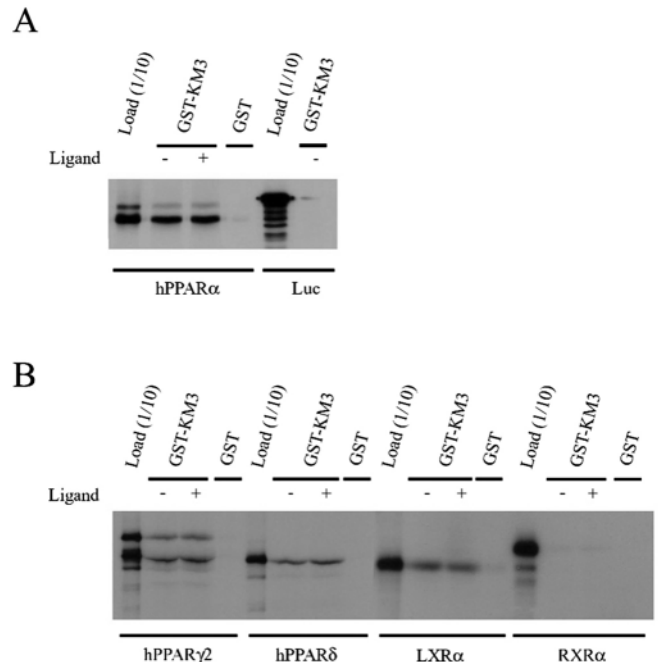

Fig. 3. CAP350 interacts with PPARs and LXRα, but not RXRα, in vitro. (A) Human PPARα and firefly luciferase (Luc) as a control for nonspecific protein binding, and (B) PPARγ2, PPARδ, LXRα and RXRα synthesized in vitro and labeled with L-[^{35}S]methionine were incubated with immobilized GST-KM3 or GST alone in the presence or absence of cognate ligand, as indicated. Bound radiolabeled proteins were analyzed by SDS-PAGE. The ligands used were Panagonist B for PPARs, 22-[R]-hydroxycholesterol for LXRα, and 9-cis retinoic acid for RXRα. Lanes designated Load (1/10) had 10% of the L-[^{35}S]methionine-labeled protein added to each of the respective binding assays.

was unable to bind to GST-KM3 (binding efficiency of less than 0.05%).

To determine the specificity of the CAP350/PPARα interaction, we tested the binding of CAP350 to other nuclear hormone receptors (Fig. 3B). PPARγ$_2$, PPARδ and liver-X-receptor α (LXRα) all interacted with GST-KM3 both in the absence and the presence of their respective ligands. In contrast, the 9-cis retinoic acid receptor RXRα was unable to bind to GST-KM3 either alone or in the presence of its cognate ligand, 9-cis retinoic acid. These findings indicate that CAP350 can physically and specifically interact with PPARα and other related nuclear hormone receptors in vitro.

CAP350 redirects the subcellular and subnuclear localization of PPARα

CAP350 contains a CAP-Gly motif, a conserved glycine-rich domain found in several cytoskeletal-associated proteins including restin, dynactin and *Drosophila* Glued (Riehemann and Sorg, 1993). The subcellular localization of CAP350, however, has not been reported. Indirect immunofluorescence studies using NIH3T3 cells transfected with expression vectors for PPARα and/or human CAP350 tagged with the c-Myc epitope (hereafter called CAP350) were carried out to determine the subcellular distribution of CAP350 and its potential colocalization with PPARα. To help ensure that any observed effects were not simply the result of the overexpression of exogenous protein, we used cells in which plasmids were non-replicating, transfected cells with low amounts of DNA, observed cells at early time points posttransfection (18-24 hours) and used an imaging system specifically designed for low light sensitivity. Cells transfected with plasmid expressing CAP350 and stained by indirect immunofluorescence with anti-c-Myc antibody showed that CAP350 was present in the nucleus and concentrated in distinct subnuclear foci (Fig. 4A panels D-F). CAP350 nuclear inclusions varied in number from four to nine spots per cell, were approximately 1 μm in diameter, and appeared to be evenly spheroid as determined by three-dimensional (3D) reconstruction. Nuclear spots were the first structures visible at the onset of CAP350 expression and could be observed at the limit of detection as early as 18 hours posttransfection. CAP350 was also present around the nucleus, in the centrosome (see below) and in the cytoplasm in association with a branched filamentous network. Other than an overall increase in expression level, there were no notable changes in the intracellular distribution of CAP350 with increased expression times. These findings are consistent with the prediction that CAP350 is a centrosome- and cytoskeleton-associated protein. Immunoblot analysis of NIH3T3 cells transfected with CAP350 expression plasmid showed the synthesis of a predominant, immunoreactive protein of approximately 350 kDa, as expected for full-length human CAP350, and no significant proteolytic breakdown products were observed (data not shown).

Cells transfected with PPARα and immunostained with anti-PPARα antibody showed that PPARα was present exclusively in the nucleus in a diffuse pattern (Fig. 4A panels A-C). PPARα was not detected in nucleoli or in the cytoplasm. This subcellular distribution pattern is consistent with recent findings using transfected GFP-PPARα fusion proteins
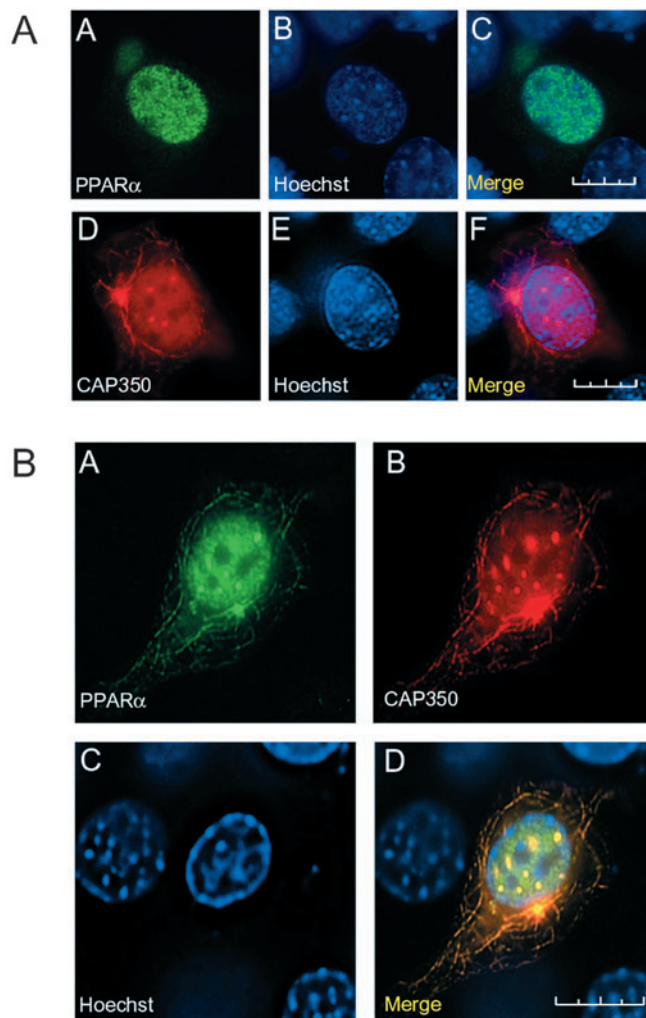
Fig. 4. CAP350 redirects PPARα subcellular localization. (A) NIH3T3 cells were transfected separately with expression plasmids for PPARα (panels A-C) or CAP350 (panels D-F) and subjected to immunostaining with anti-PPARα antibody (green) or anti-c-Myc antibody (red) to detect tagged CAP350, as indicated. Nuclei were stained with Hoechst. Panels C and F show the respective merged images. Representative images are shown. PPARα is found distributed throughout the nucleus and is absent from the cytoplasm. CAP350 is present in the nucleus and concentrates in discrete subnuclear foci, as well as in the perinuclear region and on cytoskeletal microfilaments. (See also movie 1 in supplementary material.) (B) NIH3T3 cells were cotransfected with expression vectors for PPARα and CAP350, which were visualized in the same cell. Panel D is the merged image in which colocalization of PPARα and CAP350 is shown in yellow. (See also Movie 2 in supplementary material.) Bars, 10 μm.

(Akiyama et al., 2002). In contrast, the subcellular distribution of PPARα was found to be dramatically altered in the presence of coexpressed CAP350. PPARα was observed by immunofluorescence microscopy in subnuclear bodies, the perinuclear region and in association with cytoplasmic filaments when coexpressed with CAP350 (Fig. 4B panels A,C). This PPARα-specific immunofluorescence pattern was coincident with the CAP350 immunofluorescence signal (Fig. 4B panel B), as shown by the pseudocolored yellow signal

resulting from the merged image of the respective red and green signals of the immunostained images (Fig. 4B panel D). CAP350-mediated relocalization was specific for PPARα, since the subcellular distribution of PPARα was not altered in the presence of coexpressed CAP350 (Fig. 5A-F). To further
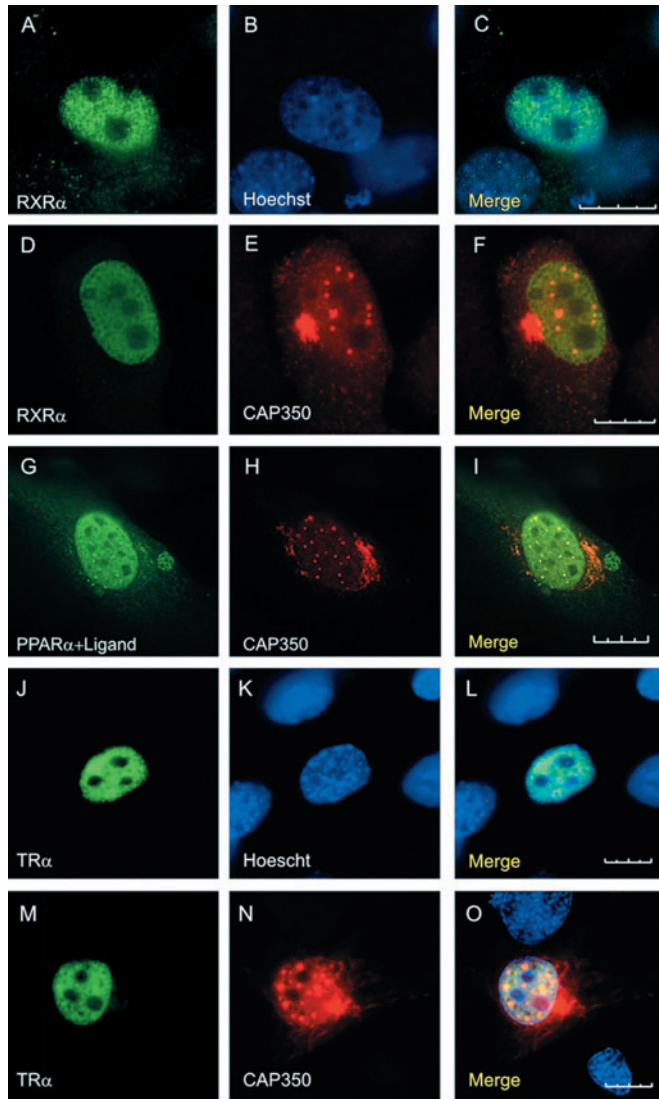

Fig. 5. CAP350 does not affect RXRα or TRα subcellular localization. Representative images of NIH3T3 cells transfected with an expression vector for RXRα alone (A-C) or TRα alone (J-L) or cotransfected with expression vectors for RXRα and CAP350 (D-F) or TRα and CAP350 (M-O), and analyzed by indirect immunofluoresence microscopy with anti-RXRα antibody or anti-TRα antibody (green) and anti-c-Myc antibody (red) to detect tagged CAP350. The merged images indicate that CAP350 does not alter the subcellular localization of RXRα or TRα (F and O, respectively). Nuclei were detected by staining with Hoechst (B and K).
(G-I) PPARα ligand does not affect the subcellular relocalization of PPARα in the presence of CAP350. NIH3T3 cells were transfected with expression vectors for PPARα and CAP350 in the presence of the PPARα ligand Panagonist B and subjected to immunostaining with anti-PPARα antibody (green) and anti-c-Myc antibody (red) to detect tagged CAP350. The merged image (I) shows that ligand does not alter the colocalization of PPARα and CAP350. Bars, 10 μm.

confirm the selectivity of CAP350 for PPARα, we also tested the effect of CAP350 expression on the localization of the nuclear hormone receptor, TRα. TRα was exclusively nuclear, as expected, and CAP350 coexpression did not affect its distribution (Fig. 5J-O). In addition, the presence of cognate ligand did not alter the distribution of PPARα in the presence of CAP350 (Fig. 5G-I) or in its absence (data not shown). These results indicate that CAP350 associates with PPARα in vivo and serves to redirect PPARα to discrete subnuclear foci and to structures within the cytoplasm. These cytoplasmic structures include the perinuclear region, with the centrosome, and branched filamentous structures within the cytoplasm.

To determine if the strong immunofluorescence signal for CAP350 in the perinuclear region represents the centrosome, cells were transfected with plasmid expressing CAP350 and immunostained with antibodies to pericentrin, a centrosome-specific protein (Dictenberg et al., 1998). In addition, cells expressing CAP350 were cotransfected with plasmid expressing fluorescent EYFP-α-tubulin, another centrosome structural component. The perinuclear region containing CAP350 also stained with both pericentrin and EYFP-α-tubulin (Fig. 6B panels A-C and Fig. 6C panels A-C, respectively), confirming that CAP350 is in fact present in the centrosome.

The Golgi complex and the centrosome are often juxtaposed, as demonstrated in NIH3T3 cells transfected with plasmids expressing fluorescent ECFP-Golgi and CAP350 (Fig. 6A panels A-C). To exclude the possibility that CAP350 is associated with the Golgi complex, cells were treated with Brefeldin A to disrupt the Golgi complex prior to fluorescence microscopy (Fujiwara et al., 1988). Brefeldin A disrupted the Golgi complex as expected but had no effect on the CAP350 staining pattern (Fig. 6A panels D-F). This finding confirms that CAP350 is associated with the centrosome, and that PPARα is recruited to this structure in the presence of CAP350.

The cytoskeleton contains three major filament systems: actin microfilaments, tubulin-containing microtubules and intermediate filaments (IF) (Paramio and Jorcano, 2002; Strelkov et al., 2003). To determine the nature of the cytoplasmic microfilaments defined by immunodetection of CAP350 and PPARα, fluorescence colocalization studies were performed with anti-cytokeratin antibodies, which are specific for IFs in NIH3T3 cells, and with phalloidin-FITC, which is specific for actin (Prochniewicz-Nakayama et al., 1983). The fluorescent mitochondrial marker ECFP-Mito was used as a control for other cytoplasmic structures. CAP350 colocalized with cytokeratin (Fig. 7A panels A-C) but not with mitochondria (Fig. 7B, panels A-C) or actin filaments (Fig. 7B, panels D-F), indicating that the branched localization of CAP350 is due to association with IFs.

As reported above, PPARα colocalized with CAP350 in discrete subnuclear foci (see Fig. 4B panels A-D). The cell nucleus is a highly compartmentalized organelle containing multiple morphologically distinct subnuclear bodies that perform various spatially and temporally regulated biological functions (Hendzel et al., 2001). Subnuclear bodies appear as speckles, which are sites of concentration of pre-mRNA splicing factors, Nup98 bodies, coiled-coil bodies and promyelocytic leukemia PML/ND10 bodies, among others (Hendzel et al., 2001; Doucas, 2002; Griffis and Powers, 2003). To address the nature of the PPARα/CAP350-associated
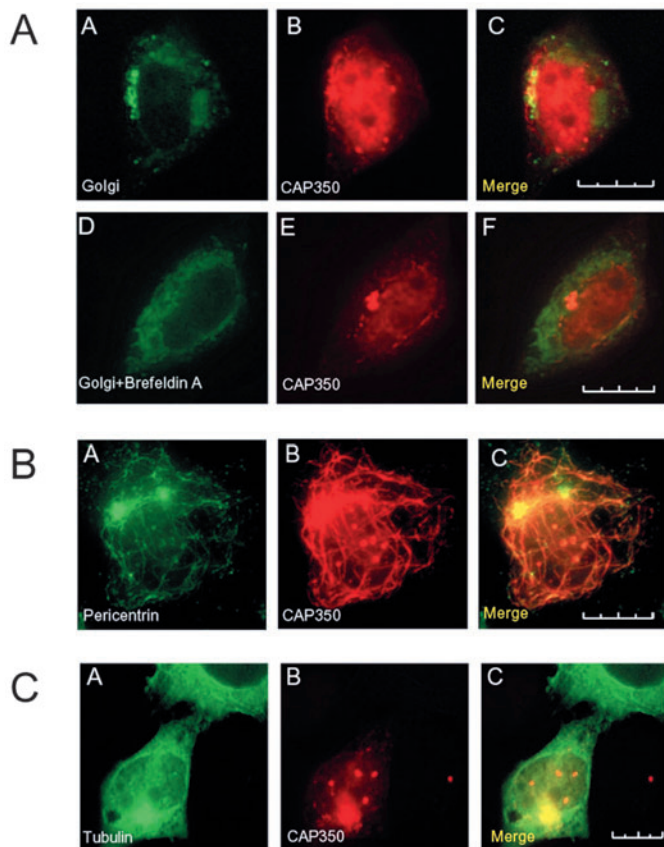
Fig. 6. CAP350 colocalizes with the centrosome. (A) NIH3T3 cells were cotransfected with expression plasmids for CAP350 and ECFP-Golgi in the absence (panels A-C) or presence (panels D-F) of Brefeldin A. CAP350 was detected by immunostaining with anti-c-Myc antibody (red). The merged images show that CAP350 does not colocalize with the Golgi complex. (B) CAP350 is present in the centrosome. NIH3T3 cells were transfected with the expression plasmid for CAP350 and subjected to immunostaining with anti-pericentrin antibody (green) (panel A) and anti-c-Myc antibody (red) to detect tagged CAP350 (panel B). The merged image indicates colocalization of CAP350 with pericentrin in the centrosome, as shown by the yellow color. (See also Movie 3 in supplementary material.) (C) CAP350 colocalizes with α-tubulin. NIH3T3 cells were cotransfected with plasmids expressing EYFP-α-tubulin (panel A) and CAP350 (panel B). CAP350 was detected by immunostaining with anti-c-Myc antibody (red). The colocalization of CAP350 with EYFP-α-tubulin is seen as yellow in the merged image (panel C). Bars, 10 μm.

cytoplasmic bodies (Fig. 8E-H) (Griffis and Powers, 2003). CAP350 subnuclear bodies were also distinct from PML bodies (Fig. 8I-K). The nature of the PPARα- and CAP350-containing subnuclear foci therefore remains to be established.

In a related line of investigation, we determined whether ongoing transcription is required for colocalization of CAP350 and PPARα and/or for the formation or stability of CAP350-containing subnuclear bodies. Cells were cotransfected with plasmids expressing PPARα and CAP350 and treated with the RNA transcription inhibitor actinomycin D prior to fixation and immunostaining. The presence of actinomycin D had no effect on the pattern of PPARα/CAP350 colocalization in the nucleus or in the cytoplasm (Fig. 8M-P).

An amino-terminal domain of CAP350 recruits PPARα

CAP350 is a very large, complex protein of greater than 3000

subnuclear foci, GFP fusions to proteins that target distinct subnuclear bodies were examined for their potential colocalization with CAP350. The splicing factor SF2/ASF was located in speckles as expected, while CAP350 was excluded from these regions (Fig. 8A-D). Similarly, CAP350 subnuclear foci did not colocalize in either the nucleus or cytoplasm with Nup98, a nucleoporin involved in RNA nucleo-cytoplasmic shuttling, found both at the nuclear pore complex and in association with discrete subnuclear and

Fig. 7. CAP350 colocalizes with intermediate filaments (IF). (A) NIH3T3 cells were transfected with expression plasmid for CAP350 and subjected to immunostaining with anti-cytokeratin antibody (green; panel A) and anti-c-Myc antibody (red) to detect tagged CAP350 (panel B). The merged image (panel C) shows colocalization of CAP350 and cytokeratin, a marker of IFs, as indicated by the yellow color. (B) CAP350 does not colocalize with mitochondria. NIH3T3 cells were cotransfected with expression plasmids for ECFP-Mito (panel A) and CAP350 (panel B). CAP350 was detected by immunostaining with anti-c-Myc antibody (red). The merged image (panel C) shows that CAP350 does not colocalize with mitochondria. (C) CAP350 does not colocalize with actin filaments. NIH3T3 cells transfected with expression plasmid for CAP350 were analyzed by staining with phalloidin-FITC to detect actin (green) (panel A) and anti-c-Myc antibody (red) to detect CAP350 (panel E). The merged image (panel C) shows that CAP350 does not colocalize with actin filaments. Bars, 10 μm.
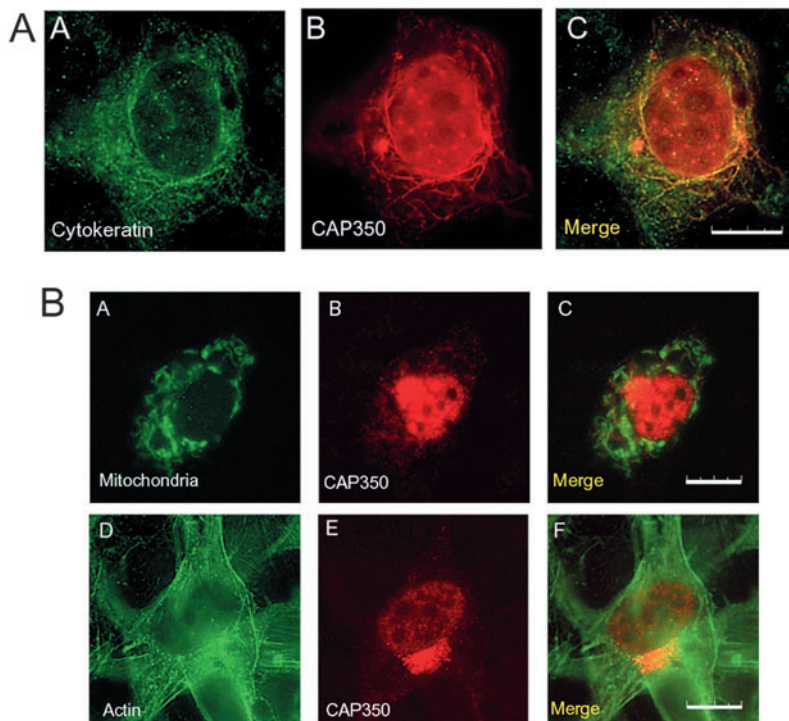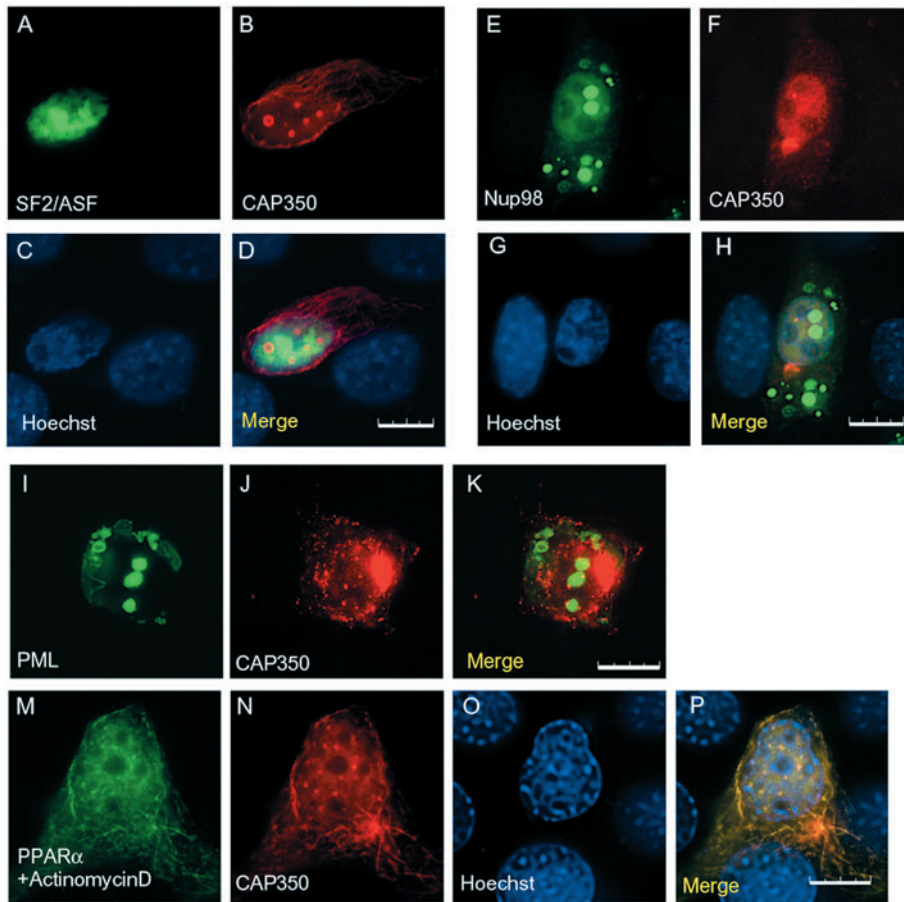
Fig. 8. CAP350 and PPARα colocalize to novel subnuclear foci. NIH3T3 cells were cotransfected with expression plasmids for CAP350 and GFP-SF2/ASF (A-D), GFP-NUP98 (E-H), or GFP-PML (I-K), as indicated. CAP350 was detected by immunostaining with anti-c-Myc antibody (red). Nuclei were detected by staining with Hoechst. The respective merged images indicate that the CAP350-containing subnuclear foci do not represent speckles, Nup98 bodies or PML bodies. Formation of CAP350-PPARα subnuclear foci does not require active transcription. NIH3T3 cells were cotransfected with plasmids expressing CAP350 and PPARα in the presence of the transcriptional inhibitor, actinomycin D (M-P). CAP350 and PPARα were detected by immunostaining with anti-c-Myc antibody (red) and anti-PPARα antibody (green), respectively. Nuclei were detected by staining with Hoechst. The merged image (P) indicates that the colocalization of CAP350 and PPARα in subnuclear bodies does not require ongoing RNA synthesis. Bars, 10 μm.

are sufficient for its subnuclear localization but lack determinants for its targeting to other subcellular sites. To determine if the amino-terminal 890 amino acids of CAP350 are sufficient to recruit PPARα to subnuclear sites, we cotransfected pEYFP-CAP350$_{1-890}$ together with the vector pmRFP-PPARα expressing an mRFP-PPARα fusion protein. mRFP-PPARα colocalized with EYFP-CAP350$_{1-890}$ to discrete subnuclear foci (Fig. 9A-C). Therefore, the first 890 amino acids of CAP350 are sufficient to direct CAP350 to subnuclear foci, but not other subcellular sites, and to recruit PPARα.

Nuclear hormone receptor-interacting partners often contain one or more copies of the sequence LXXLL, a conserved motif that mediates protein-protein interactions (Glass and Rosenfeld, 1999; Qi et al., 2002). The first 890 amino acids of CAP350 contain a single putative LXXLL signature motif (LSHLL, amino acids 759-763). To determine if this motif is required for CAP350/PPARα colocalization in vivo, the leucines at positions 762 and 763 within the 1-890 CAP350 subfragment were changed to alanine by site-directed mutagenesis of its encoding cDNA, and the subcellular location of an EYFP-tagged version of this mutant fragment, EYFP-CAP350$_{1-890(LSHAA)}$, was determined in transfected NIH3T3 cells. EYFP-CAP350$_{1-890(LSHAA)}$ retained its ability to accumulate in subnuclear spots like the fluorescent wild-type fragment EYFP-CAP350$_{1-890}$; however, the mutant chimera was unable to recruit PPARα to these spots (Fig. 9D-F). Thus, the integrity of the LSHLL motif in CAP350$_{(1-890)}$ is necessary for its colocalization with PPARα in vivo and its accumulation in subnuclear foci. To determine if this motif was required for direct CAP350/PPARα physical association, we carried out in vitro binding assays between PPARα and GST fusions to CAP350$_{1-890}$ or the LXXLL mutant derivative CAP350$_{1-890(LSHAA)}$. As expected, the 1-890 residue CAP350 fragment was able to efficiently bind to PPARα in vitro (Fig. 9G), indicating that this amino-terminal region contains determinants necessary for its direct interaction with PPARα. Surprisingly, PPARα bound equally well to the mutant form of the CAP350 fragment (Fig. 9G). These findings suggest that the integrity of the LXXLL motif is not necessary for the direct interaction of CAP350 with PPARα and that this motif must play some other role in the recruitment of PPARα to subnuclear spots.

CAP350 antagonizes PPARα-dependent transactivation

To explore any functional consequences of CAP350/PPARα

amino acids. CAP350 is probably a multifunctional protein that contains a variety of functional domains, which serve various roles; however, there is at present little knowledge of its structural and functional properties. We have shown that amino acids 572-1176 of CAP350 are sufficient for its interaction with PPARα in both the yeast two-hybrid system (Miyata et al., 1996) and in vitro (see Fig. 3A). To begin to define the subregions of CAP350 that determine its subcellular localizations and/or its association with PPARα in mammalian cells, we performed live cell imaging of NIH3T3 cells transfected with a plasmid, pEYFP-CAP350$_{1-890}$, expressing a chimera of yellow fluorescent protein (YFP) and the amino-terminal 890 amino acids of CAP350 (Fig. 9). EYFP-CAP350$_{1-890}$ localized exclusively to subnuclear foci and not to the centrosome or cytoplasmic structures (Fig. 9A). The subcellular localization of full-length EYFP-CAP350 was indistinguishable from that of CAP350 (data not shown), indicating that the amino-terminal 890 amino acids of CAP350

interaction on transactivation by PPARα, we examined the effects of CAP350 expression on PPARα-mediated induction of a PPRE-linked reporter gene by transient transfection analysis of H4IIEC3 cells, a rat hepatoma cell line that is responsive to peroxisome proliferators (Zhang et al., 1992; Zhang et al., 1993). H4IIEC3 cells were transfected with the reporter plasmid pTK-PPRE(×3)-*Luc*, which contains three copies of the PPRE of the rat gene encoding the peroxisomal β-oxidation bifunctional enzyme (Zhang et al., 1993). Incubation of transfected cells in the presence of the PPARα 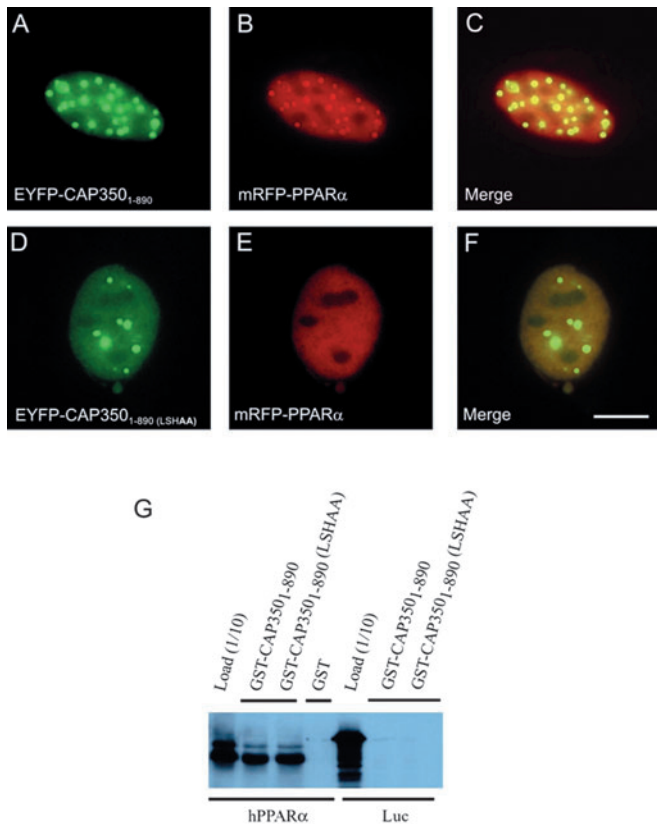activator Wy-14,643 led to a specific sevenfold induction in reporter gene activity over basal levels (Fig. 10A), consistent with a previous report (Zhang et al., 1992). Addition of increasing amounts of the expression vector for CAP350 led to a dose-dependent inhibition of transcriptional activity. At the highest concentration of CAP350 expression plasmid, Wy-14,643-mediated transcriptional induction was completely repressed. Inhibition of transcription by CAP350 was specific to the PPRE-containing reporter gene construct, since the expression of CAP350 had no effect on the basal transcriptional activity of the control reporter plasmid pTK-*Luc*, which lacks a PPRE (data not shown).
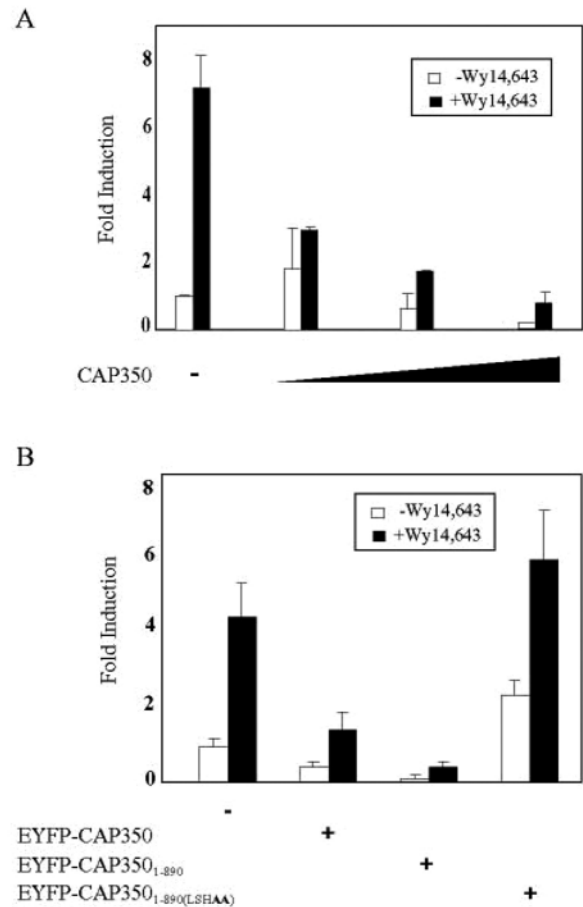

Fig. 9. An amino-terminal fragment of CAP350 localizes to subnuclear foci and recruits PPARα to these foci. NIH3T3 cells were cotransfected with expression plasmids for EYFP-CAP350$_{1-890}$ (green) and mRFP-PPARα (red) (A-C) or for EYFP-CAP350$_{1-890(LSHAA)}$ and mRFP-PPARα (D-F) and subjected to live cell imaging. Panels C and F show the merged images of panels A and B and panels D and E, respectively. Representative images are shown. EYFP-CAP350$_{1-890}$ and EYFP-CAP350$_{1-890(LSHAA)}$ concentrate in subnuclear foci but are not present in the centrosome or in cytoskeletal microfilaments. mRFP-PPARα colocalizes in subnuclear foci with EYFP-CAP350$_{1-890}$ but not with EYFP-CAP350$_{1-890(LSHAA)}$ (panel F). Bar, 10 μm. (G) Mutation of the LXXLL motif in the amino-terminal fragment of CAP350 does not abrogate its binding to PPARα in vitro. In vitro synthesized, *L*-[^{35}S]methionine-labeled PPARα or firefly luciferase (Luc) (a control for nonspecific protein binding) synthesized in vitro was incubated with immobilized GST-CAP350$_{1-890}$ or GST-CAP350$_{1-890(LSHAA)}$, as indicated, and bound radiolabeled proteins were analyzed by SDS-PAGE. The left lanes show parallel binding reactions carried out with labeled luciferase as a negative control. Lanes designated Load (1/10) had 10% of the *L*-[^{35}S]methionine-labeled protein added to each of the respective binding assays.

Fig. 10. CAP350 antagonizes transcriptional transactivation by endogenous PPARα in vivo. (A) H4IIEC3 cells were transfected with the reporter plasmid pTK-PPRE(×3)-*Luc* in the presence or absence of the PPARα ligand Wy-14,643 (100 μM) and increasing amounts of the CAP350 expression vector pMT-hCAP350 (0-0.5 μg/plate), as indicated. Luciferase activity was measured 48 hours posttransfection. The values are the average (±s.d.) fold-induction relative to untreated cells (taken as 1) from three independent transfections carried out in triplicate and normalized for protein and the levels of expression of β-galactosidase from the plasmid pCMVLacZ used to control for transfection efficiency.(B) An amino-terminal fragment of CAP350 antagonizes PPARα-mediated transcriptional activity in an LXXLL-dependent manner. H4IIEC3 cells were transfected with the reporter plasmid pTK-PPRE(×3)-*Luc* in the presence or absence of the PPARα ligand, Wy-14,643 (100 μM) and expression vectors for EYFP-CAP350, EYFP-CAP350$_{1-890}$, EYFP-CAP350$_{1-890(LSHAA)}$ or EYFP alone (designated by the (−) symbol), as indicated. Luciferase activity was measured as above.

To confirm that the inhibition of the PPARα-mediated transcriptional transactivation by CAP350 is dependent on their interaction, we carried out transient transfection reporter assays in the presence of vectors expressing EYFP-CAP350$_{1-890}$ and EYFP-CAP350$_{1-890(LSHAA)}$ (Fig. 10B). EYFP-CAP350$_{1-890}$ inhibited Wy-14,643 induction of PPRE reporter gene activity in H4IIEC3 cells as effectively as EYFP-CAP350 containing the full-length CAP350. In contrast, EYFP-CAP350$_{1-890(LSHAA)}$ was unable to inhibit PPARα-mediated transcriptional transactivation, consistent with the observation that this mutant derivative of CAP350 is unable to direct colocalization with PPARα in vivo. These findings indicate that CAP350 negatively regulates transcriptional transactivation by endogenous PPARα in vivo, and does so in part by sequestering PPARα in subnuclear foci.

Discussion

Accumulating evidence indicates that intracellular trafficking and dynamic changes in the subcellular distribution and compartmentalization of type II nuclear hormone receptors play an important role in their biological functions (Baumann et al., 1999; Baumann et al., 2001b; Hager et al., 2002). In contrast to previous assumptions that most type II nuclear hormone receptors are constitutively present in the nucleus, studies using live cell imaging have shown that these receptors are mobile and can shuttle between the cytoplasm and the nucleus (Barsony and Prufer, 2002; Baumann et al., 2001a; Baumann et al., 2001b). Moreover, receptors may continuously exchange between subnuclear macromolecular complexes. The mechanisms that mediate these processes and their biological significance are largely unknown. In this report, we have identified CAP350 as a centrosome-associated protein and a previously undescribed interacting partner of PPARα and have shown that CAP350 recruits PPARα to discrete subcellular and subnuclear compartments. CAP350 was also shown to inhibit transactivation by PPARα in vivo. CAP350 interacts with PPARδ, PPARγ and LXRα, but not with RXRα, suggesting that CAP350 plays a general role in the biological functions of PPARα and related nuclear hormone receptors.

Nuclear compartmentalization plays an important role in gene regulation, and many transcription factors, including steroid and nuclear hormone receptors such as the glucocorticoid, estrogen, thyroid and retinoic acid receptors, and nuclear receptor coregulatory molecules such as GRIP-1, SMRT, SRC-1, and RIP140, have been shown to accumulate in discrete foci distributed throughout the nucleoplasm (Carmo-Fornesca, 2002; Doucas, 2002; Hendzel et al., 2001; Zilliacus et al., 2001; Tazawa et al., 2003; van Steensel et al., 1995). The identity, composition and function of these foci remain to be elucidated. Our findings show that CAP350 directs nuclear relocalization of PPARα from a diffuse nuclear distribution to discrete CAP350-containing foci. These foci were shown to be distinct from PML/ND10 bodies, nuclear speckles and Nup98 bodies. It has been suggested that nuclear hormone receptor-containing subnuclear foci may represent sites of transcriptional activity, since in some cases their formation is dependent upon the presence of cognate ligand (Htun et al., 1996). However, this does not appear to be the case for PPARα, as CAP350-dependent relocation of PPARα was unaffected by the presence of exogenous PPARα ligand,

although the presence of endogenous ligand cannot be excluded. Moreover, the RNA synthesis inhibitor actinomycin D had no effect on CAP350-mediated subnuclear redistribution of PPARα, suggesting that PPARα containing foci do not represent sites of active transcription or that their formation requires ongoing transcription. An alternative explanation is that foci serve to regulate the local or global concentration of regulatory molecules in the nucleus and/or represent domains for the assembly/disassembly of multicomponent transcription complexes (Hendzel et al., 2001). In some cases, subnuclear foci may function to inactivate factors, perhaps by sequestering them from active transcription sites and/or promoting their degradation. Our findings that CAP350-dependent localization of PPARα in subnuclear foci correlates with an inhibition of PPARα-mediated transcriptional transactivation are consistent with such a scenario. We showed that the first 890 amino acids of CAP350 were sufficient to localize CAP350 to subnuclear foci, to recruit PPARα to these sites, and to inhibit transcriptional transactivation by PPARα. Importantly, a CAP350 mutant derivative harboring a change in the LSHLL nuclear hormone receptor-binding motif, while still able to localize to subnuclear foci, was unable to recruit PPARα or to inhibit its transcriptional activity. Surprisingly, PPARα was still able to interact with this mutant form of the CAP350 subfragment in vitro. Thus, while the LXXLL motif is required for the colocalization of PPARα and CAP350 in nuclear bodies in vivo, the integrity of this motif is not necessary for direct physical interaction between the two proteins, suggesting that the amino-terminal subfragment of CAP350 must harbor other determinants that mediate its interaction with PPARα. Our findings suggest that the LXXLL motif serves some other role in the colocalization of CAP350 and PPARα to subnuclear bodies and in the repression of transactivation by PPARα, perhaps through its recruitment of auxiliary proteins that mediate these events.

Our findings also provide the first demonstration that PPARα can localize to distinct compartments in the cytoplasm. Prominent among these is the CAP350-dependent recruitment of PPARα to the centrosome. The centrosome, consisting of the centrioles and pericentriolar components, is a dynamic structure that is involved in microtubule organization and mitotic spindle formation, and it acts as a structural and regulatory scaffold for numerous cellular processes such as mitosis, cytokinesis, cargo and vesicle transport, regulated proteolysis and cell signaling (Doxsey, 2001). The functional significance of the association of numerous regulatory molecules that control diverse cellular processes with the centrosome is unclear. The centrosome, through associated proteins such as pericentrin and protein kinase A-anchoring proteins, may serve as a central scaffold that coordinates the recruitment of regulatory factors to other subcellular locations (Rempel, 2001). Our findings demonstrate for the first time that a nuclear hormone receptor can localize to the centrosome through protein-protein interactions with CAP350. An intriguing possibility is that the centrosome controls the proteolytic degradation of PPARα. PPARα is expressed in a circadian rhythm, and its protein levels are tightly controlled in the cell, in part through ubiquitination and subsequent proteolysis via the 26S proteasome (Blanquart et al., 2002; Hauser et al., 2002). Recent evidence indicates that the centrosome contains a functional 26S proteasome that

degrades ubiquitinated proteins (Fabunmi et al., 2002; Wigley et al., 1999), raising the possibility that the recruitment of PPARα to the centrosome regulates PPARα turnover.

PPARα also colocalized with CAP350 in a branched filamentous network throughout the cytoplasm. These filaments were identified as IFs by virtue of the colocalization of CAP350 with cytokeratin, a major component of IFs. IFs are responsible for the mechanical integrity of the cell and are involved in a number of cellular processes such as motility, transport and cell signaling (Paramio and Jorcana, 2002; Stelkov et al., 2003). The IF cytoskeleton interacts with a large number of proteins, including kinases, phosphatases and motor proteins such as dynactins and kinesins. Evidence suggests that IFs are involved in the transmission of cell signals between the membrane, cytoplasm and nucleus, and in the movement of macromolecules within the cell. The biological significance of the association of PPARα with IFs remains to be elucidated, but this association provides further evidence that the cellular localization of PPARα is a dynamic process that potentially involves the distribution or exchange of PPARα among multiple subcellular and nuclear sites through protein-protein interactions. The result of these intracellular dynamics could be to modulate biological activity by compartmentalization. Ongoing studies using live cell imaging and fluorescent protein fusions will resolve many of these questions.

In summary, we have identified a novel PPARα-interacting protein, CAP350, that has revealed new and unexpected findings related to the intracellular distribution of this important nuclear hormone receptor. The subcellular localization of CAP350 and redistribution of PPARα imply that the PPARα/CAP350 complex is dynamic. We have also shown that CAP350 inhibits PPARα-mediated transcriptional transactivation in vivo and that this inhibition correlates with CAP350-mediated recruitment of PPARα to subnuclear foci. Whether the cellular functions of PPARα are also modulated by CAP350 through the distribution of PPARα to other subcellular locations awaits further experiments. Our findings identify for the first time a cellular function for CAP350 as a potential regulator of PPARα action, and reveal new dimensions to the diversity of processes involved in the biological functions of PPARα and perhaps other nuclear hormone receptors.

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