PPARγ is a member of the PPAR subfamily of nuclear receptors. In this work, the structure of the human PPARγ cDNA and gene was determined, and its promoters and tissue-specific expression were functionally characterized. Similar to the mouse, two PPAR isoforms, PPARγ1 and PPARγ2, were detected in man. The relative expression of human PPARγ was studied by a newly developed sensitive competitive polymerase chain reaction method, which allowed us to distinguish between PPARγ1 and γ2 mRNA. In all tissues analyzed, PPARγ2 was much less abundant than PPARγ1. Adipose tissue and large intestine have the highest levels of PPARγ mRNA; kidney, liver, and small intestine have intermediate levels; whereas PPARγ is barely detectable in muscle. This relative expression of PPARγ in colon warrants further study in view of the well-established role of fatty acid and arachidonic acid derivatives in colonic disease. Similarly as mouse PPARγs, the human PPARγs are activated by thiazolidinediones and progestin J and bind with high affinity to a PPRE. The human PPARγ gene has nine exons and extends over more than 100 kilobases of genomic DNA. Alternate transcription start sites and alternate splicing generate the PPARγ1 and PPARγ2 mRNAs, which differ at their 5'-ends. PPARγ1 is encoded by eight exons, and PPARγ2 is encoded by seven exons. The 5'-untranslated sequence of PPARγ1 is comprised of exons A1 and A2, whereas that of PPARγ2 plus the additional PPARγ2-specific N-terminal amino acids are encoded by exon B, located between exons A2 and A1. The remaining six exons, termed 1 to 6, are common to the PPARγ1 and γ2. Knowledge of the gene structure will allow screening for PPARγ mutations in human metabolic disorders, whereas knowledge of its expression pattern and factors regulating its expression could be of major importance in understanding its biology.

White adipose tissue is composed of adipocytes, which play a central role in lipid homeostasis and the maintenance of energy balance in vertebrates. These cells store energy in the form of triglycerides during periods of nutritional affluence and release it in the form of free fatty acids at times of nutritional deprivation. Excess of white adipose tissue leads to obesity (1–3), whereas its absence is associated with lipodystrophic syndromes (4). In contrast to the development of brown adipose tissue, which mainly takes place before birth, the development of white adipose tissue is the result of a continuous differentiation/development process throughout life (2, 5). During development, cells that are pluripotent become increasingly restricted to specific differentiation pathways. Adipocyte differentiation results from coordinate changes in the expression of several proteins, which are mostly involved in lipid storage and metabolism, that give rise to the characteristic adipocyte phenotype. The changes in expression of these specialized proteins are mainly the result of alterations in the transcription rates of their genes.

Several transcription factors including the nuclear receptor PPARγ (6, 7), the family of CCAAT enhancer binding proteins (C/EBP) (8–13) and the basic helix-loop-helix leucine zipper transcription factor ADD1/SREBP1 (14, 15) orchestrate the adipocyte differentiation process (for reviews, see Refs. 1, 3, 16–18). In contrast to the wide tissue distribution of the various C/EBPs, PPARγ has been shown to have an adipose-restricted pattern of expression in mouse. The currently favored hypothesis is that C/EBPβ and δ induce the expression of PPARγ (11), which then triggers the adipogenic program. Terminal differentiation then requires the concerted action of both PPARγ, C/EBPα, and ADD1/SREBP1 (7, 15). Several arguments support the important role of PPARγ in adipocyte differentiation. First, overexpression of PPARγ by itself can induce adipocyte conversion of fibroblasts (6). In addition, PPARγ together with C/EBPα can induce transdifferentiation of myoblasts into adipocytes (19). Second, the description of functional PPREs in the regulatory sequences of several of the genes that are induced during adipocyte differentiation, such as the genes coding for adipocyte fatty acid binding protein, aP2 (6), phosphoenolpyruvate carboxykinase (PEPCK) (20), acyl-CoA synthetase (ACS) (21, 22), and lipoprotein lipase (LPL) (23), is consistent with the crucial role attributed to PPARγ in adipocyte differentiation. Finally, PPAR activators, such as

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To isolate genomic P1-derived artificial chromosome (PAC) clones containing the entire human PPARγ gene, the primer pair LF-3 and LF-14 was used to amplify an 86-bp probe with human genomic DNA as template. This fragment was then used to screen a PAC human genomic library from human foreskin fibroblasts. Three positive clones, P-8854, P-8855, and P-8856, were isolated. Restriction digestion and Southern blotting were performed according to classical protocols as described by Sambrook et al. (36). Sequencing reactions were performed, according to the manufacturer instructions, using the T7 sequencing kit (Pharmacia Biotech Inc.).

**Experimental Procedures**

**Materials and Oligonucleotides**

The oligonucleotides used for various experiments in this manuscript are listed in Table I.

**Tissue Biopsies and Cell Culture**

Omental adipose tissue, small and large intestine, kidney, muscle, and liver biopsies were obtained from non-obese adult subjects undergoing elective surgery or endoscopy. All subjects had given informed consent, and the project was approved by the ethics committee of the University of Lille. All tissue was immediately frozen in liquid nitrogen until RNA preparation. Standard cell culture conditions were used to maintain 3T3-L1 (obtained from ATCC), CV-1 (a kind gift from Dr. R. Evans, Salk Institute, La Jolla, CA), and Hep G2 cells (ATCC). BRL-49,653, supplied by Ligand Pharmaceuticals, San Diego, CA (in DMSO) and fatty acids (in ethanol) were added to the medium at the concentrations and times indicated. Control cells received vehicle only. Fatty acids were-
Fig. 1. Sequence of the human PPARγ cDNA and comparison with the mouse PPARγ sequence. A, sequence comparison of mouse and human PPARγ. Identical amino acids are indicated by a vertical line and conservative changes are indicated by a dot. B, splicing of exon A2 and exon B with exon 1. hPPARγ1 contains two extra amino acids relative to mPPARγ1. The presence of a promoter in front of exon A and B is indicated by an arrow. Nucleotides in capitals are located in exons, whereas the nucleotides in the intron are in lowercase.
FIG. 2. RT-competitive PCR method to measure PPARγ mRNA levels. A, scheme highlighting the features of the vector pSG5hPPARγ and the vector derived from it, pBSCompPPARγ, which served to synthesize competitor DNA. The primer pairs used in the RT and PCR reactions...
plexed to serum albumin contained in delipidated and charcoal-treated fetal calf serum by preincubation for 45 min at 37 °C.

**mRNA Analysis by RT-Competitive PCR Assay**

RNA preparation of total cellular RNA was performed as described previously (37). The absolute mRNA concentration of the differentially spliced PPARγ-variants was measured by reverse transcription reaction followed by competitive polymerase chain reaction (RT-competitive PCR) in the presence of known amounts of competitor DNA yielding amplicons of different size allowing the separation and the quantification of the PCR products. The competitor was constructed by deletion of a 74-bp fragment (nucleotides 943 to 1016) containing the primer pair 5'-TTCTCCCGTAAATGGGAGGC-3' (nt +146 to +165) or to the B son (285.5'-CGATTCCTCTACTGACTAC-3', nt +41 to +59). Therefore, the same competitor served to measure either total PPARγ mRNAs (γ1 + γ2; with primers γAS and γS) or, specifically, PPARγ2 mRNA (with primers γAS and γS28). The γAS/γS primer pair gave PCR products of 474 and 400 bp for the PPARγ mRNAs and competitor, respectively. The primer pair γAS/γS28 gave 580 bp for PPARγ2 mRNA and 506 bp for the competitor. For analysis of the PCR products, the sense primers γS and γS25 were 5'-end labeled with the fluorescent dye Cy-5 (Eurogentec, Belgium).

First-strand cDNA synthesis was performed from total RNA (0.1 μg) in the presence of the antisense primer γAS (15 pmol) and of thermostable reverse transcriptase (2.5 units; Tth DNA polymerase, Promega) as described (38). After the reaction, half of the RT volume was added to the PCR mix (90 μl) containing either the primer pair γAS/γS for the assay of PPARγ total mRNA, whereas the other half was added to a PCR mix (10 μl Tris-Cl, pH 8.3, 100 mM KCl, 0.75 mM EDTA, 5% glycerol, 0.2 mM dNTP, 5 units of Taq polymerase) containing the primer pair γAS/γS25 for the assay of PPARγ2 mRNA. Four aliquots (20 μl) of the mixture were then transferred to microtubes containing a different, but known, amount of competitor. After 120 s at 95 °C, the samples were subjected to 40 PCR cycles (40 s at 95 °C, 50 s at 95 °C, and 50 s at 72 °C). The fluorescence-labeled PCR products were analyzed by 4% denaturing polyacrylamide gel electrophoresis using an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia, Uppsala, Sweden), and integration of the area under the curve using the Fragment manager software (Pharmacia) was performed as described (38).

To validate this technique, human PPARγ2 mRNA was synthesized by in vitro transcription from the expression vector pBS5hPPARγ (Riboblock system, Promega) and quantified by competitive PCR over a wide range of concentrations (0.25–25 attomole (amol) added in the RT reaction). Standard curves obtained when assaying PPARγ-total mRNA or PPARγ2 mRNA are shown in Fig. 2C. The linearity (r = 0.99) and the slopes of the standard curves (0.98 and 1.11) indicated that the RT-competitive PCR was quantitative and that all the mRNA molecules are copied into cDNA during the RT step. For the lower limit of the assay was about 0.05 amol of mRNA in the RT reaction, and the interassay variation of the RT-competitive PCR was 7% with six separated determinations of the same amount of PPARγ mRNA.

**Western Blot Analysis of PPARγ**

Cells and tissues were homogenized in a lysis buffer of PBS containing 1% Triton X-100 (Sigma). Tissues were homogenized in extraction buffer containing PBS and 1% Nonidet P-40 (Sigma), 0.5% sodium deoxycholate (Sigma), 0.1% SDS (Sigma). Fresh mixture protease inhibitor (ICN) was added (100 mg/ml AEGSF, 5 mg/ml EDTA, 1 mg/ml leupeptin, 1 mg/ml pepstatin). Protein extracts were obtained by centrifugation of the lysate at 4 °C, and concentration was measured with the Bio-Rad DC Protein colorimetric assay (Bio-Rad, Hercules, CA).

Protein (100 μg) was separated by SDS-PAGE, transferred to nitrocellulose membrane (Amersham Life Science, Inc.), and blocked overnight in blocking buffer (20 μl Tris, 100 μl NaCl, 1% Tween-20, 10% skim milk). Filters were first incubated for 4 h at room temperature with rabbit IgG anti-mPPARγ (10 mg/ml), raised against an N-terminal PPARγ peptide (amino acids 20–104), and next developed for 1 h at room temperature with a goat anti-rabbit IgG (whole molecule) peroxidase conjugate (Sigma) diluted at 1/500. The complex was visualized with 4-chloro-1-naphtol as reagent.

**Analysis of Promoter Activity**

To test the activity of the human PPARγ promoters several reporter constructs were made. A 1-kb fragment of PAC clone 8856 was isolated by PCR using the oligonucleotides LF-35 (binding antisense in the PPARγ2 5'-UTR) and the oligonucleotide LF-58 (binding sense at position -1000 of the PPARγ2) was sequenced, and was inserted into EcoRV site of pBluescript (Stratagene, La Jolla, CA). After digestion of plasmid pBSγ2p1000 with SmaI and PstI, the insert was cloned into the reporter vector pGL3 (Promega), creating the expression vector pGL3γ2p1000. To isolate the PPARγ1 promoter, an 8-kb EcoRI fragment, which hybridized with the oligonucleotide LF-2 (corresponding to as well as the different-sized amplicons obtained are indicated. B, typical analysis of the fluorescence-labeled PCR products on an automated fluorescence DNA sequencer using a denaturing 4% polyacrylamide gel electrophoresis. C, validation of the RT-competitive PCR assay and standard curves obtained when assaying PPARγ total mRNA or PPARγ2 mRNA. The linearity (r = 0.99) and the slopes of the standard curves (0.98 and 1.11) indicated that the RT-competitive PCR is really quantitative and that all the mRNA molecules are copied into cDNA during the RT step.
the 5′-UTR of γ1, was cloned into pBlueScript. Partial mapping and sequencing of this clone revealed the presence of a 3-kb fragment upstream of the transcription initiation site. To test for promoter activity, a SacI/XhoI digestion of this clone containing the 3-kb promoter was inserted in the same sites of pGL3, resulting in the final vector pGL3γ1p3000. The pSG5-haPPARγ (39) and pMSV-C/EBPα (10) expression vectors were described elsewhere. Transfections were carried out in 60-mm plates using standard calcium phosphate precipitation techniques (for 3T3-L1, CV-1, and COS cells) (22). Luciferase and β-galactosidase assays were carried out exactly as described previously (22).

Electrophoretic Mobility Shift Assays (EMSA) and Oligonucleotide Sequences

haPPARγ (39), hPPARγ2, and mRXRα (40) proteins were synthesized in vitro in rabbit reticulocyte lysate (Promega). Molecular weights and quality of the in vitro translated proteins were verified by SDS-PAGE. PPAR (2 μl) and/or RXR (2 μl) were incubated for 15 min on ice in a total volume of 20 μl with 1-ng probe, 2.5 μg of poly(dI-dC) and 1 μg of herring sperm DNA in binding buffer (10 mM Tris-HCl pH 7.9, 40 mM KCl, 10% glycerol, 0.05% Nonidet P-40, and 1 mM dithiothreitol). For competition experiments, increasing amounts (from 10- to 200-fold molar excess) of cold oligonucleotide (AII-J-PPRE, 5′-GATCCTTCAAC-TCCCGAACGTGACCTTTGTCCTGGTCCC-3′) were included just before adding T4-PNK end-labeled AII-J-PPRE oligonucleotide. DNA-protein complexes were separated by electrophoresis on a 4% polyacrylamide gel in 0.25 × TBE buffer at 4 °C (43).

RESULTS

Cloning of the human PPARγ cDNA—A cDNA probe containing a 200-bp (KpnI-BgIII) fragment encoding the DNA binding domain of the mouse PPARγ (44) was used to screen a human adipose tissue cDNA library. Several independent human PPARγ cDNA clones, representing both the PPARγ1 and PPARγ2 subtypes, were isolated and sequenced (Fig. 1A). The human PPARγ protein shows a 99% similarity and a 95% identity on the amino acid level with mouse PPARγ (Fig. 1A). Interestingly, the initiation codon for human PPARγ1 is different from the mouse PPARγ1 (Fig. 1B). Therefore, human PPARγ1 is 2 amino acid residues longer than its mouse homologue.

Expression of PPARγ mRNA and Protein—To analyze the expression pattern of the two PPARγ isoforms, we developed a sensitive RT-competitive PCR assay in which relative amounts of PPARγ1 and γ2 mRNA could be measured from minute quantities of RNA (0.1 μg total RNA). This method relies on the co-amplification in the same tube of known amounts of competitor DNA (Fig. 2A) with PPARγ cDNA, obtained after reverse transcription from total tissue RNA. The competitor and the target used the same fluorescently labeled PCR primers but yield amplicons with a different size (Fig. 2, A and B), allowing their separation and quantification on an automated sequencing gel at the end of the reaction (Fig. 2C). All tissue preparations were carefully dissected, and the RNA was shown to be free of contamination with adipose tissue as evidenced by the absence of human leptin mRNA by RT-competitive PCR assay (38) (data not shown). PPARγ1 mRNA was the predominant PPARγ isoform in all human tissues analyzed (Fig. 3). PPARγ2 was detected in both liver and adipose tissue where it accounted for 15% of all PPARγ mRNA. Interestingly, in addition to the high level of expression of PPARγ mRNA expected in adipose tissue, we found a very high level of PPARγ1 in large intestine. In contrast to adipose tissue, large intestine contained no PPARγ2 mRNA. Kidney, liver, and small intestine contained intermediate levels of PPARγ mRNA, whereas PPARγ1 mRNA was barely detectable in skeletal muscle (Fig. 3).

Next, the expression of the human PPARγ protein was analyzed in human adipose tissue. A PPARγ specific antibody, raised against a peptide corresponding to amino acids 20–104 of mPPARγ, was used. This antibody is highly specific for PPARγ and does not cross-react with PPARα and δ in Western blot experiments (Fig. 4, A and B). Using this antibody in a Western blot of protein extracts from human adipose tissue, we detected a band (potentially representing a doublet) with an approximate molecular mass of 60 kDa, consistent with the predicted mass of PPARγ1 and 2 and with the protein product generated by in vitro transcription/translation in the presence of [35S]methionine (Fig. 4, A and B).

PPARγ2 Binds and Transactivates through a PPRE—To an-
analyze whether PPARγ could bind to a PPRE, classically composed of direct repeats spaced by one intervening nucleotide (DR-1), EMSA was performed using in vitro transcribed/translated PPARγ2 protein. An oligonucleotide containing a high affinity PPRE, previously identified in the apoA-II promoter J site, was used in EMSA (29). This oligonucleotide was capable of binding both human and hamster PPARγ/mRXRa heterodimers in EMSA (Fig. 5, lanes 5 and 6). Homodimers of either hPPARγ or mRXRa, however, were incapable of binding to this oligonucleotide. When increasing concentrations of unlabeled apoA-II J site were added as competitor, binding of the hPPARγ/mRXRa heterodimer to the labeled PPRE was almost completely inhibited (Fig. 5, lanes 7–9). In addition, oligonucleotides corresponding to the PPRE elements of the ACO or LPL genes competed, albeit less efficiently (Fig. 5, compare lanes 7 with 10 and 13).

We next verified that the human PPARγ2 cDNA was capable of activating gene transcription through a PPRE. Therefore, 3T3-L1 preadipocytes were cotransfected with the PPARγ2 expression vector pSG5hPPARγ2 and a PPRE-driven luciferase reporter gene. The luciferase gene was under the control of a multimerized ACO-PPRE site and the TK promoter (Fig. 6). hPPARγ2 was capable of activating this PPRE-based reporter 2-fold, an effect which was substantially enhanced when hPPARγ2 was cotransfected together with RXRa. Upon the addition of the PPARγ ligand BRL-14653, luciferase expression was increased 6-fold when the transfection was done with hPPARγ2 alone or at least 10-fold when the cells were cotransfected with both hPPARγ2 and mRXRa. Similar results were obtained when prostaglandin J2 was used as a PPARγ ligand (data not shown).

Characterization of the Transcription Initiation Site of the Human PPARγ Gene—To unambiguously identify the 5'-end of the cDNA, several approaches were undertaken. First, primer extension experiments were performed, utilizing different human adipose tissue RNA samples, and results were independently confirmed by using 5'-RACE. Several primer extension products were seen for the PPARγ1 mRNA using primer LF2.
The nucleotides in the exon are indicated in uppercase letters, whereas the flanking nucleotides in the intron are in lowercase. The approximate size of the introns are indicated in kilobases, and the exact length in base pairs of the exons are indicated between brackets. Amino acids encoded by the nucleotides flanking the intron/exon border are indicated by their letter symbol. The stop codon is indicated by an asterisk.

### Table II

| Exon (bp) | Donor | Intron in kb | Acceptor | Exon |
|-----------|-------|--------------|----------|------|
| A1 (97)   | GCCAG gtcagagt... | >20 | ...ttgtaag ATTIG | A2 |
| A2 (74)   | TACCG gtaagtaa... | >20 | ...ccccctcag AA ATG | 1 |
| B (211)   | CAA G gttaggtt... | 21 | ...ccccctcag AA ATG | E |
| 1 (231)   | CAA G gtatagtg... | 1.6 | ...atcacag GT GCA | Q |
| 2 (170)   | C AAG gtaattaa... | 9.5 | ...cttgtcag GGT T | M |
| 3 (139)   | AAG G gtatagtaa... | 10.7 | ...ctctatag CC ATC | K |
| 4 (203)   | A TCA gttagtctc... | 10 | ...attgcag CCA T | S |
| 5 (451)   | GGA G gtagattg... | 16.3 | ...ttccccccag AC CGC | I |
| 6 (248)   | TAC TAG cagaga... |  | ...ttccccag AC CGC | 6 |

One major extension product of 62 bp was observed consistently with the primer LF-35 for PPARγ2. A second extension product of 96 bp was found using the same primer (Fig. 7, B and C). The results of 5'-RACE were consistent with the primer extension (Fig. 7B). The transcription initiation sites identified correlated well with the transcription initiation sites observed for the mouse PPARγ2 mRNA (45). A striking feature of the human PPARγ2 5'-UTR is its high degree of sequence conservation with the mouse 5'-UTR (see Fig. 9). It awaits further study to determine the exact implications of this conservation.

### Structural Organization of the Human PPARγ Gene

To clone the human PPARγ gene and to determine its promoter sequence, we screened a PAC human genomic library derived from human foreskin fibroblasts. Three positive clones (P-8854, P-8855, and P-8856), each spanning from human to PPARγ2, were isolated. All three clones were then shown to hybridize with the oligos LF-14 (corresponding to exon B) and LF-36 (exon 6), which indicates that they span most of the PPARγ coding region. More importantly, clone P-8856 also hybridized to oligo LF-2 and, hence, contains the transcription initiation site for PPARγ1 and 2. This clone was further characterized by Southern blotting and partial sequence analysis, which allowed the construction of a physical map of the human PPARγ locus (Fig. 8). The human PPARγ gene spans more than 100 kb. The PPARγ1 and PPARγ2 mRNAs are encoded by 8 and 7 exons, respectively. The 5'-untranslated region of the PPARγ1 mRNA is encoded by two exons, which we, in analogy to the nomenclature used for the mouse gene, named exon A1 and A2. The coding region of PPARγ1 is contained in the next six exons (exons 1 to 6). Exons 1 to 6 also encode the majority of PPARγ2 mRNA. The additional 28 amino acids of PPARγ2 as well as the 5'-UTR are encoded by the B exon, which is located between exons A2 and A1.

The length of the introns was determined by long-range PCR (CLONTech Tth polymerase mix) using the oligonucleotide pairs LF-3/LF-18, LF-20/LF-21, LF-22/LF-23, LF-24/LF-25, LF-26/LF-27, and LF-28/LF-29 and the PAC clone P-8856 as a template. The intron-exon boundaries were sequenced using genomic DNA as template. The 5' donor and 3' acceptor splice sites were found to be conforming to the consensus splice donor and acceptor sequences (Table II). The DNA binding domain of the receptors is encoded by exons 2 and 3, each encoding a separate zinc finger. The entire ligand binding domain is encoded by exons 5 and 6, which are separated by 16.3 kb of intron sequence.

### Discussion

Two important findings recently underlined the importance of the PPARγ transcription factor. First, PPARγ has been iden-
Fig. 9. Sequence of the proximal promoter of the human and mouse PPARγ1 (A) and γ2 (B) genes. The relevant consensus binding sites of transcription factors (TATA sequence, the GC-rich sequences, the C/EBP, and AP-1 consensus sites) are indicated by boldface letters. Numbering is relative to the transcription initiation site. The * indicates the transcription initiation site of PPARγ1.
The Human PPARγ Gene

**Fig. 10. Tissue-specific activity of the PPARγ promoter.** A, normalized luciferase activity of the pGL3-γ1p3000 construct containing 3000 bp of regulatory sequence of the human PPARγ gene after transfection in 3T3-L1 and Hep G2 cells. Transfections were performed as described under “Experimental Procedures.” Scheme of the reporter constructs pGL3-γ1p3000 used in transfection assays is shown above the graphic. B, normalized luciferase activity of the pGL3-γ2p1000 construct containing 1000 bp of regulatory sequence of the human PPARγ gene after transfection in 3T3-L1 and Hep G2 cells. Transfections were performed as described under “Experimental Procedures.” Scheme of the reporter constructs pGL3-γ2p1000 used in transfection assays is shown above the graphic.

tified as one of the key factors controlling adipocyte differentiation and function in rodent systems (6, 7). Second, the recent identification of prostaglandin J2 derivatives and antidiabetic thiazolidinediones as natural and synthetic PPARγ ligands, respectively (28, 29, 46–48). Thiazolidinediones are a new group of anti-diabetic drugs which improve insulin-resistance (for review, see Refs. 49 and 50). The identification of thiazolidinediones as PPARγ ligands together with the central role that adipose tissue plays in the pathogenesis of important metabolic disorders, such as obesity and non-insulin-dependent diabetes mellitus (NIDDM), have generated a major interest to determine the role of this PPAR subtype in normal and abnormal adipocyte function in humans.

The PPARγ gene spans about 100 kb and is composed of 9 exons, which give rise to PPARγ1 and PPARγ2 mRNAs by differential promoter usage and differential splicing. The gene structure as well as the sequence of the encoded protein are well conserved between human and mouse (45) (99% similarity and 95% identity). Relative to the mouse, hamster, and Xenopus PPARγ (6, 39, 51), the human protein contains two additional amino acids. This is in agreement with the previous reports on the human PPARγ cDNA (34, 35, 52). The availability of the structure of the human PPARγ gene and protein will now allow for genetic studies, evaluating its role in disorders such as insulin resistance, NIDDM, and diseases characterized by altered adipose tissue function such as obesity or lipodystrophic syndromes.

To determine tissue-specific patterns of expression of the human PPARγ gene, we developed an RT-competitive PCR assay. Unlike results of previous reports, which used commercially available kits or single RNA samples (34, 35), we used multiple independent samples to base our conclusions on. As was observed in rodents (6, 7), we found PPARγ to be strongly expressed in adipose tissue. In addition to adipose tissue, the large intestine had high levels of PPARγ expression. Several other tissues, such as liver, kidney, and small intestine contained lower but nevertheless considerable levels of PPARγ RNA. Skeletal muscle, in contrast, contained only trace amounts of PPARγ mRNA.

In adipose tissue and liver, about 15% of all PPARγ mRNA was of the PPARγ2 type, whereas in the remaining tissues no PPARγ2 mRNA was detected. These observations have several important implications. First, our data question the relative importance of PPARγ2. Indeed, our results in humans as well as the data by Xue et al. (53) in rodent adipocytes show consistently lower levels of PPARγ2 mRNA and protein relative to the PPARγ1 subtype. These observations are in line with the previous observations that the N-terminal domain of PPARγ was dispensable, both regarding transcriptional activation and capacity to induce adipocyte differentiation in vitro (7). However, the N-terminal domain is highly conserved between different species, suggesting it might have an important function in vivo. Second, PPARγ expression is much more widespread than previously realized, which implies that PPARγ controls gene expression in several tissues in addition to adipose tissue. Especially striking is the high level of PPARγ expression in the human large intestine. These reports are consistent with the reported high level expression of PPARγ in colonic mucosa in mouse (54). It is interesting to note that fatty acids, potential PPAR activators, have been shown to play an important role in modulating the function of the large intestine. For instance diets enriched in saturated lipids have been shown to predispose to the development of colon cancer (55). Furthermore, it has been shown that diets enriched in ω-3 fatty acids, powerful PPAR activators, have a beneficial response on inflammatory diseases of the gastrointestinal tract such as colitis ulcerosa and Crohn’s disease (56, 57). Since the high level expression of PPARγ suggest that it might play an important role in normal and abnormal colonic function, further studies aimed at exploring this are definitely needed. Finally, the low levels of PPARγ expression in skeletal muscle cells also deserve some reflection. Muscle is responsible for clearance of the majority of glucose in the body and abnormal muscle glucose uptake is one of the prime features of insulin resistance and NIDDM. The low levels of PPARγ in muscle argue, therefore, that the beneficial effects of thiazolidinedione antidiabetic agents are not likely to
be due to a direct effect of these agents on PPARγ present in the muscle. In fact, even though the liver has considerably higher levels of PPARγ relative to muscle, thiazolidinediones do not seem to affect PPAR responsive genes in liver tissue at the concentrations commonly used to lower glucose levels (23). This observation together with the observed tissue distribution of PPARγ suggests that the glucose lowering effects of the thiazolidinedione PPARγ ligands are primarily a result of their activity on adipose tissue, which then, via a secreted signal, might influence muscle glucose uptake.

To identify the molecular circuitry underlying tissue-specific expression of PPAR, we cloned and performed an initial characterization of the human PPARγ promoters. As shown, 3000 bp of the PPARγ1 and 1000 bp of the PPARγ2 promoter account for substantial levels of basal promoter activity. Further functional studies are underway to determine elements necessary for tissue-specific and regulated expression of the PPARγ gene. In this context, it will be interesting to determine the effects of transcription factors known to induce adipocyte differentiation on PPARγ expression in this tissue and to define the hierarchical role that PPARγ plays in this process. PPARγ is not the only transcription factor involved in adipocyte differentiation. In addition to PPARγ, the basic helix-loop-helix leucine zipper factor ADD-1/SREBP1 and transcription factors of the C/EBP family also play a role in determining adipocyte differentiation. It is interesting to note that, as in the mouse PPARγ2 promoter (45), a potential consensus C/EBP response element could be identified in the human PPARγ2 promoter by homology searches. This observation fits well with the previous observation that forced expression of C/EBPβ could induce PPARγ expression and further studies on this subject are underway (11, 12).

In conclusion, we report the characterization of the human PPARγ gene structure and furthermore define the structure of the PPARγ1 and γ2 promoter. In addition, our data show that human PPARγ has a similar structure and similar transactivation function as the rodent PPARs. The expression patterns of PPARγ1 and γ2 show that in man, PPARγ1 is the preminent form. Our results furthermore demonstrate that, in addition to adipose tissue, human colon expresses high levels of PPARγ. It is expected that the gene structure will facilitate our analysis of eventual PPARγ mutations in humans, whereas knowledge of expression patterns and sequence elements, as well as factors regulating PPARγ gene expression, could be of major importance in understanding PPAR biology.

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