The Transcriptional Response to a Peroxisome Proliferator-activated Receptor α Agonist Includes Increased Expression of Proteome Maintenance Genes*§

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The nuclear receptor peroxisome proliferator-activated receptor α (PPARα), in addition to regulating lipid homeostasis, controls the level of tissue damage after chemical or physical stress. To determine the role of PPARα in oxidative stress responses, we examined damage after exposure to chemicals that increase oxidative stress in wild-type or PPARα-null mice. Primary hepatocytes from wild-type but not PPARα-null mice pretreated with the PPAR pan-agonist WY-14,643 (WY) were protected from damage to cadmium and paraquat. The livers from intact wild-type but not PPARα-null mice were more resistant to damage after carbon tetrachloride treatment. To determine the molecular basis of the protection by PPARα, we identified by transcript profiling genes whose expression was altered by a 7-day exposure to WY in wild-type and PPARα-null mice. Of the 815 genes regulated by WY in wild-type mice (p ≤ 0.001; ±1.5-fold or ≤ -1.5-fold), only two genes were regulated similarly by WY in PPARα-null mice. WY increased expression of stress modifier genes that maintain the health of the proteome, including those that prevent protein aggregation (heat stress-inducible chaperones) and eliminate damaged proteins (proteasome components). Although the induction of proteasomal genes significantly overlapped with those regulated by 1,2-dithiole-3-thione, an activator of oxidant-inducible Nrf2, WY increased expression of proteasomal genes independently of Nrf2. Thus, PPARα controls the vast majority of gene expression changes after exposure to WY in the mouse liver and protects the liver from oxidant-induced damage, possibly through regulation of a distinct set of proteome maintenance genes.

Peroxisome proliferators (PPs)1 are a large class of structurally heterogeneous pharmaceutical and industrial chemicals

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The abbreviations used are: PP, peroxisome proliferator; D3T, 1,2-dithiole-3-thione; H&E, hematoxylin and eosin; HSF, heat shock factor; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; WY, WY-14,643; RT, reverse transcription; GO, gene ontology; FC, -fold change; WT, wild type.
Expression of some heat shock proteins is essential for cellular survival under physical or chemical exposure conditions that increase oxidative stress (11). Regulation of the Hsp genes by heat or chemical-induced oxidative stress is controlled by heat shock factors (HSFs) activated under conditions in which the level of unfolded proteins increase.

The proteasome carries out ubiquitin-dependent and -independent proteolysis of damaged proteins (12). The 26 S proteasome consists of a 20 S core containing 14 different subunits and two 19 S regulatory particles containing an additional 14 subunits. Proteasomal (Psm) gene expression can be induced by treating cells with proteasomal inhibitors (13) or by the indirect antioxidant 1,2-dithiole-3-thione (D3T), which activates the oxidant-inducible transcription factor Nrf2 (NF-E2-related factor 2) (14). Like PPARα, activation of Nrf2 protects cells from different forms of stress (15). In addition to the Psm genes, Nrf2 regulates genes involved in phase II xenobiotic metabolism through the antioxidant response element. Nrf2 is negatively regulated by Keap1, an actin-binding protein that sequesters Nrf2 in the cytoplasm. Indirect antioxidants such as the dithiolthionines (e.g. D3T) cause the dissociation of Nrf2 from Keap1, allowing nuclear accumulation of Nrf2 and enhanced expression of cytoprotective genes. Studies using Nrf2-null or Keap1/Nrf2-null mice have shown that Nrf2 is a key sensor regulating the expression of genes promoting cell survival (15). One possible mechanism that may contribute to the ability of Nrf2 and dithiolthionines to increase cell survival after toxicant exposure is the increased expression of Psm genes through antioxidant response elements in their promoter regions (16).

Using oligonucleotide arrays, we examined the extent of PPARα-dependent gene expression changes after WY-14,643 (WY) exposure in the livers of wild-type and PPARα-null mice. To help identify stress-inducible genes, we compared the WY profiles with those from two treatments that induce genes involved in tissue protection. We found that PPARα regulates a large battery of genes involved in maintenance of the proteome and that these genes partially overlap with genes regulated by stress-inducible transcription factors, Nrf2 and HSF. Consistent with this, hepatocytes from PPARα-null mice exhibit decreased resistance to oxidative stress induced by chemical exposure.

**Experimental Procedures**

**Animals and Treatments**—Wild-type and PPARα-null mice 9–12 weeks of age on an SV129 background were used in these studies. The mice were originally obtained from Dr. Frank Gonzalez, NCI, National Institutes of Health. Control and treated mice were provided with NIH-07 rodent chow (Zeigler Brothers, Gardeners, PA) and deionized, filtered water ad libitum. Lighting was on a 12-h light/dark cycle. Male mice were given by gavage each day for 3 days the PPAR pan-agonist WY-14,643 (ChemSyn Science, Lenexa, KS) at 50 mg/kg/day. Male mice were also fed a control diet or a diet containing WY in the diet (500 ppm) WY-14,643 (ChemSyn Science, Lenexa, KS) at 50 mg/kg/day. Male mice were sacrificed at 6 h after the single dose or 24 h after the last of seven consecutive doses. Male Nrf2-null mice or corresponding wild-type mice were treated with one dose of D3T (0.5 mmol/kg) and sacrificed 24 h later (14). For the Nrf2 nuclear localization experiments, male B6C3F1 mice were given one or seven consecutive daily doses of WY (50 mg/kg) by gavage as described above. Mice were either sacrificed at 6 h after the single dose or 24 h after the last of seven consecutive doses. Male Nrf2-null mice or corresponding wild-type mice on an ICR genetic background were given seven consecutive doses of WY by gavage as described above and sacrificed 24 h after the last dose. Portions of the livers were rapidly snap frozen in liquid nitrogen and stored at −70 °C until analysis. Where appropriate, slices of liver were fixed in 10% neutral buffered formalin for 48 h, transfixed to 70% ethanol, and embedded in paraffin. 5-μm sections were cut and mounted on slides and stained with H&E. H&E-stained liver sections were examined by light microscopy. All animal studies were conducted under federal guidelines for the use and care of laboratory animals and were approved by Institutional Animal Care and Use Committees.

**Conditions for Culturing and Treatment of Primary Hepatocytes**—Primary cultures of mouse hepatocytes were obtained using a two-step collagenase perfusion method as described by Keddleris and Held (17). Viability was determined using trypan blue exclusion. Preparations over 85% viable were used for cell culture. Cells were plated in wells that had been treated with rat tail collagen, using Williams media E containing 10% fetal bovine serum, l-glutamine, penicillin, and streptomycin. After 2 h, the media were changed to serum-free media containing insulin, transferrin, and selenium (Invitrogen), l-glutamine, penicillin, streptomycin, and dexamethasone. The cultures were left overnight at 37 °C and 5% CO2. Cell cultures were treated the following morning with Me2SO or WY (50 μM final concentration). After 24 h, the cells were also treated with the indicated concentrations of pararatum or compound to induce oxidative stress with the same solvent WY in the media. Cells and media were harvested 24 h after treatment. The lactate dehydrogenase assay was used to assess damage as described (17).

**RNA Isolation and Analysis of Gene Expression**—For the analysis of gene expression by oligonucleotide arrays, liver tissue from three mice was analyzed from each treatment group. Liver RNA was isolated using a modified guanidium isothiocyanate method (TRIzol®; Invitrogen) and chloroform. RNA purity and quality were assessed by ethidium bromide staining followed by resolution on denaturing agarose gels and also by the RNA 6000 LabChip® kit using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). RNA integrity was assessed by ethidiuim bromide staining. The quality of the RNA. Subsequently, the same samples were hybridized to Murine GeneChip® U74Av2 GeneChips (Affymetrix). All procedures were carried out according to the manufacturer’s recommendations using the antibody amplification technique. Images were initially processed using MAS version 5.0 software (Affymetrix). Hybridization quality was assessed by visual inspection of the image and from a report generated by MAS version 5.0. Criteria for an acceptable hybridization included: background <100; noise (RawQ) <3; 3-fold change for select housekeeping genes. Hybridizations not meeting these criteria were repeated, beginning at the target preparation step. The data were analyzed and statistically filtered using Rosetta Resolver® version 3.0 software (Rosetta Inpharmatics, Kirkland, WA). The threshold for significance for these experiments was set at p ≤ 0.001 and ≥1.5- or ≥−1.5-fold change, and the relative increase or decrease in mRNA abundance was reported as in relative to wild-type or null controls. Genes were grouped into functional classes using Gene Ontology (available on the World Wide Web at www.geneontology.org) identifiers found in the U74Av2 template (version available August 1, 2003 on the World Wide Web at www.affymetrix.com). The functional categories and their corresponding gene ontology (GO) numbers were as follows: peroxisome biogenesis, 7031; lipid transport, 6869; fatty acid metabolism, 6631 and 6635; fatty acid biosynthesis, 6833; glutathione transferase, 4364; glutathione conjugation, 6803; monoxygenase activity, 4497; response to heat, 9408; translational elongation, 6414; initiation factor, 5855 and 5852; translation initiation, 6413; chaperone, 3754; protein folding, 6457; diaulfide isomerase, 3755; ubiquitin-dependent protein catabolism, 6511; proteasome, 501 and 4299. To compare expression of the genes in each functional class between treatment groups, we first eliminated genes that were expressed at low levels (<300; ~5% of the average gene expression in wild-type mouse liver) and not altered by compound treatment in any group. Expression changes were averaged over all genes in that GO category. Categories that had <10% of all genes altered were eliminated. The average number of genes altered in each gene category was 21%. Categories were visualized by TreeView (18). Identification of expressed sequence tags was facilitated by euGenes (available on the World Wide Web at iubio.bio.indiana.edu:89/mouse/). Identification of potential binding sites for PPARα in proteasome genes was made using the transcription Element Search System (available on the World Wide Web at www.cbl.uehenn.edu/tesa/). Sequences upstream of the start codon for each gene were retrieved from the World Wide Web at genome.ucsc.edu/index.html?org=Mmuse). 4 kb of the promoter region was analyzed for each gene using the consensus PPRE sequence 5'-TGACCTnTGACCTnAGTTT-3'.

**Real-Time RT-PCR Analysis**—The levels of expression of the selected genes were quantified using real-time RT-PCR analysis. Briefly, total RNA was extracted as described above and reverse transcribed with...
murine leukemia virus reverse transcriptase and oligo(dT) primers. The forward and reverse primers for selected genes (Supplementary Table 1) were designed using Primer Express software, version 2.0 (Applied Biosystems, Foster City, CA). The SYBR green DNA PCR kit (Applied Biosystems) was used for real time PCR analysis. The relative differences in expression between groups were expressed using cycle time (Ct) values as follows. The Ct values of the genes were first normalized with β-actin of the same sample, and then the relative differences between control and treatment groups were calculated and expressed as relative increases, setting the control as 100%. Assuming that the Ct value is reflective of the initial starting copy and that there is 100% efficiency, a difference of one cycle is equivalent to a 2-fold difference in starting copy.

Nuclear Localization of Nrf2—Hepatic nuclear extracts were isolated from liver homogenates according to Dignam et al. (19). SDS-PAGE was performed by loading 25 μg of nuclear extract on a 6% SDS-polyacrylamide gel. Following separation, gels were transferred to nitrocellulose membranes (Amersham Biosciences), and immunoblotting was carried out using a commercial anti-Nrf2 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Immunoblotted membranes were developed using the ECL Western blotting system (Amersham Biosciences) according to the manufacturer’s instructions.

Statistical Analysis of Data—Means and S.E. (n = 4) for RT-PCR data were calculated by Student’s t test. The level of significance was set at p ≤ 0.05. The expression ratios between WY treatment and controls and between WT mice and Nrf2-null mice were used for comparisons. Spearman rank correlation test was performed using SAS (SAS version 6.12, SAS Institute, Research Triangle Park, NC).

RESULTS

PPARs Alters Responses to Chemical-induced Stress—We hypothesized from previous studies that hepatocytes from PPARα-null mice would be more sensitive to chemical-induced oxidative damage. To test this hypothesis, we examined the effect of chemicals that induce protein damage through increases in oxidative stress. In in vitro studies, primary hepatocytes isolated from wild-type or PPARα-null mice were pretreated with WY or Me2SO followed by co-treatment with WY or Me2SO and either paraquat or cadmium, inducers of oxidative stress. Damage after chemical exposure was assessed by leakage of lactate dehydrogenase. WY exposure itself did not induce cell damage. Hepatocytes from Me2SO-treated, wild-type, or PPARα-null mice exhibited extensive damage after exposure to paraquat or cadmium (Fig. 1, A and B). Damage induced by either compound was significantly suppressed by pretreatment with WY in wild-type but not in PPARα-null mice.

We examined the effect of chemical treatment in the intact mouse. Wild-type and PPARα-null mice were exposed to carbon tetrachloride (CCL4), an inducer of centrilobular necrosis in mice and rats. The active species is a free radical form that induces extensive lipid and protein damage (20). Mice were given one intraperitoneal injection of 250 μl of CCL4 per kg of body weight. After 2 days of treatment, all male PPARα-null mice and 40% of the female PPARα-null mice either died or were moribund, whereas only a quarter of the male wild-type mice and none of the female wild-type mice exhibited outward signs of toxicity. The characteristic centrilobular necrosis was also more severe in male PPARα-null mice and included bridging necrosis between central veins (Fig. 2 A). Necrosis after CCL4 exposure in male PPARα-null mice was observed at lower doses than wild-type mice. At higher doses, the extent of damage was approximately the same in the two strains (Fig. 2 B).

These studies indicate that PPARα determines the extent of damage to agents that induce increases in oxidative stress.

PPARα Is Required for the Majority of Transcriptional Changes Altered by WY—To uncover the gene regulatory networks under control of PPARα that may contribute to alteration of chemical-induced damage, we examined the transcript profiles in the livers of wild-type and PPARα-null mice exposed to WY for 7 days. In wild-type mice, WY altered 6.5% of the genes queried, including 434 up-regulated and 381 down-regulated genes. WY had almost no effect in PPARα-null mice. Only two genes were altered by WY in PPARα-null mice, namely Cyp4a10 (10.0-fold increase in wild type versus 9.5-fold in PPARα-null) and Cyp4a14 (15.7-fold increase in wild type versus 4.5-fold in PPARα-null). Only one gene (serum amyloid A protein 4; Saa4) linked to inflammatory responses was altered in PPARα-null but not wild-type mice by WY (19.5-fold induction). The fact that there were so few parallel changes in both wild-type and PPARα-null mice was not due to the stringency of our -fold change cut-off. When we examined significantly altered genes (p ≤ 0.001) without prior filtering for -fold change, only Ugdh (1.5-fold increase in wild-type versus 1.4-fold increase in PPARα-null) and Acox1 (2.4-fold increase in wild-type versus 1.2-fold increase in PPARα-null) exhibited similar expression behavior in both strains. Acox1 was induced in PPARα-null mice treated by a PPARβ and a PPARγ agonist but not by WY (21). These results demonstrate that PPARα mediates the vast majority of transcriptional changes after WY exposure in the mouse liver.

Alteration of Proteome Maintenance Genes by PPARα—As preliminary experiments indicated altered regulation of heat stress-inducible genes by PPARα, we compared the WY transcript profiles with transcript profiles in which heat stress-
inducible genes involved in tissue protection were altered including after a 40-min 42 °C heat stress or after exposure to D3T, an antioxidant compound that indirectly activates Nrf2 (15). Transcript profiles were compared directly using Spearman correlation as a measure of similarity between groups. The correlation coefficients were statistically significant between WY in wild-type mice and D3T in either wild-type or Keap1/Nrf2-null mice (Table I), indicating that D3T alters a set of overlapping genes through a mechanism that is at least partly Keap1/Nrf2-independent. There was also a significant overlap in the profiles altered by WY and heat stress in wild-type mice. The correlation became insignificant between WY and heat stress in PPARα-null mice, indicating that PPARα controls the expression of heat stress-regulated genes that determine similarity with the WY transcript profile.

To facilitate comparison of the different treatments, the altered genes were grouped into GO functional groups as described under “Experimental Procedures.” In this analysis, we included those genes altered in wild-type and PPARα-null mice after a 3-day treatment with WY. These genes have been previously characterized (22). Almost all categories of genes were regulated similarly after 3- or 7-day WY treatment. However, many categories exhibited greater responsiveness after longer exposure.

Genes involved in lipid metabolism including peroxisome biogenesis, lipid transport, fatty acid metabolism, and fatty acid biosynthesis genes were generally up-regulated by a 7-day and, to a lesser extent, 3-day WY treatment (Fig. 3), as expected (1). Genes involved in lipid metabolism and stress responses are discussed in detail in the Supplementary Materials.

Based on evidence that PPARα acts to prevent damage from chemical-induced stress, we focused on the expression behavior of genes involved in maintenance of the proteome. These genes include those that regulate translation; protein folding, including the chaperones and functionally related chaperonins; and proteolysis, including genes involved in ubiquitin tagging and elimination of damaged or excess proteins. Of the 14 genes involved in protein synthesis (GO:6414, 5851, 5852, 6413) that were regulated either by WY, D3T, or heat stress, WY increased the expression of one initiation factor (Eif3s6) and two elongation factors (Tcebf1 and 2300002G02Rik), whereas D3T increased the expression of six factors (Eifs6, Eifs7, Gspt1, Itgb4bp, Psmd7, and 3230401O13Rik), some of which were Keap1/Nrf2-independent (Fig. S2). Heat stress in PPARα-null but not wild-type mice led to an increase in one gene (Sui1-rs1) and decreases in three genes (Gspt1, Tcea1, and 2300002G02Rik).

FIG. 2. Increased sensitivity to carbon tetrachloride exposure in PPARα-null mice. A, liver damage in PPARα-null mice but not wild-type mice after exposure to 250 μl of CCl4/kg of body weight. Groups of female mice were sacrificed 48 h after dosing, and livers were assessed for damage by H&E. B, increased sensitivity of PPARα-null mice to low CCl4 concentrations. Male mice were exposed to the indicated concentrations of CCl4 (in μl of CCl4/kg of body weight) and sacrificed 48 h later. The H&E-stained livers were evaluated for necrosis by pathological examination and scoring of severity of necrosis.
Of the 29 nonredundant genes involved in protein folding (GO:3754, 6457, 3755) including 10 overlapping genes known to be regulated by heat stress (GO:9408), WY up-regulated 12 genes and down-regulated four genes (Fig. 4A). The up-regulated genes included chaperonin subunits (Cct3, Cct6a, Cct7, Hspd1, and Hspc1), heat shock proteins (Hspa1b and Hspca), GrpE-like proteins (Grpel1 and Grpel2), and miscellaneous proteins (Dnajb10, Fkbp1a, and Tbca). Two of the down-regulated genes have protein-disulfide isomerase (Pdir-pending) or peptidylprolilisomerase (Ppib) functions. D3T up-regulated a set of 10 genes in which three overlapped with those regulated by WY. Heat stress in wild-type mice up-regulated four genes and down-regulated one. Spearman comparison of the WY- and D3T-regulated genes did not show a statistically significant overlap (data not shown). This comparison indicates that WY, D3T, and heat stress regulate unique but overlapping sets of genes involved in protein folding.

A large number of genes involved in proteasomal protein degradation were altered by WY. WY up-regulated 16 and down-regulated 2 Psm genes (1.5-fold), including genes encoding components of both the 20 and 19 S particles (Fig. 4B). Additional Psm genes were significantly up-regulated by WY (p < 0.001) but did not achieve the 1.5-fold threshold for reporting; these include Psmat6 (1.5-fold change (FC) = 1.38), Psm2 (FC = 1.38), Psmd7 (FC = 1.20), and Psmd8 (FC = 1.39). D3T up-regulated an overlapping set of genes. Spearman analysis of the WY and D3T profiles showed that the overlap was statistically significant (r = 0.59; p < 0.001). WY, but not D3T, specifically down-regulated two immunoproteasomal genes (Psmb8 and Psmb10) involved in processing viral antigens for capture by the class I major histocompatibility complex and increased the expression of PA28 (Psme1), which coordinate regulates immunoproteasome assembly and is required for efficient antigen processing (23). This indicates that WY and D3T exposure may have different functional consequences on protein processing and degradation. In addition to the Psm genes, WY and D3T increased expression of Usp14, which is bound to the 26 S proteasome and might regulate protein localization and activity (24). Heat stress had little effect on proteasomal gene expression. These data indicate that WY, like D3T, coordinately increases the expression of proteasomal genes.

PPARs Regulates Stress Modifier Genes Independently of Nrf2—We examined the functional basis for the overlap in the genes regulated by PPARs and Nrf2, including those in the Psm gene family. As WY increases oxidative stress (4) and Nrf2 is activated under conditions of oxidative stress (15), we next asked whether WY exposure increases Nrf2 activation by examination of Nrf2 nuclear accumulation. A 7-day treatment of B6C3F1 mice with WY did not result in significant increases in Nrf2 nuclear accumulation in the liver (data not shown). Nuclear accumulation also did not occur after a shorter WY exposure time (6 h) when oxidative stress was not expected to be significant (data not shown).

We determined directly whether the genes that overlapped between WY and D3T were regulated by WY in an Nrf2-dependent manner. Wild-type and Nrf2-null mice were treated with WY each day for 7 days, and the expression of 34 genes in a number of functional categories was examined by RT-PCR in the livers of these mice. Direct transcriptional targets of PPARs involved in lipid metabolism and peroxisome proliferation (Acox1, Cyp4a10,
Cyp4a14, Fabp4, and Pex11a) were up-regulated in wild-type mice as expected (Table II). The PPARα gene (Ppara) was also up-regulated by WY. Surprisingly, all PPARα target genes exhibited higher levels of induction in WY-treated Nrf2-null mice (Table II, WY, Null versus WY, WT).

We examined genes involved in xenobiotic metabolism that overlapped between WY and D3T or that were altered by PP in other studies. WY up-regulated Cyp2a4 and Cyp3a11 and down-regulated Cyp2f2 and Cyp7b1 in wild-type mice. The RT-PCR behavior of Cyp2a4 was opposite of that observed on the array. Cyp2a4 and Cyp2f2 exhibited major differences in induction in Nrf2-null mice. Cyp2a4 expression was induced to higher levels, and Cyp2f2 expression was unaffected by WY in the Nrf2-null mice. Cyp7b1 was down-regulated approximately

FIG. 4. Altered expression of proteome maintenance genes by WY. Genes involved in protein folding (A) and proteasomal degradation (B) that exhibited altered regulation in one of the six treatment groups were clustered using one-dimensional clustering (similar gene behavior) and visualized usingTreeView. Genes involved in protein folding were identified with GO identifiers for chaperone (GO:3754), protein folding (GO:6457), and disulfide isomerase (GO:3755). Genes involved in proteasomal degradation were identified with GO identifiers for ubiquitin-independent protein catabolism (GO:6511) and proteasome (GO:501, 4299). Gene abbreviations and GenBank accession numbers are shown. Scales are in -fold changes.
equally in both strains. Additionally, Cyp2e1 was up-regulated by WY only in Nrf2-null mice. The phase II metabolism genes Ephx1, Ephx2, and Fmo1 were up-regulated in wild-type mice; Ephx1 was up-regulated ∼2-fold greater in Nrf2-null mice. Gsp1 was down-regulated in both strains, whereas Gst11 and Gpx1 were up-regulated in Nrf2-null mice only. Sod2 was up-regulated in both strains. The expression pattern of PXR (Nrf2β), which regulates many of these phase I and II genes, mirrored that of Cyp2e1, Gst11, and Gpx1 in that there was no change in wild-type mice but an increase in Nrf2-null mice after WY exposure.

The chaperone proteins Hsp25, Hsp60, Hsp70, and Hsp86 exhibited increased expression in wild-type and Nrf2-null mice with little difference between strains. Hsp105 was down-regulated in wild-type but up-regulated only in Nrf2-null mice. The expression of two Psm genes (Psmb4 and Psmd4) and Usp14 was up-regulated by WY in wild-type mice. In the Nrf2-null mice, the expression of all six Psm genes examined, Ubgln2, and Usp14 was elevated, and except for Usp14, the induction in the Nrf2-null mice exceeded that in wild-type mice (∼1.5–3.0-fold). The RT-PCR data confirm the regulation of stress-inducible genes by WY observed by transcript profiling. The data indicate that WY alters the expression of stress-inducible genes by an Nrf2-independent mechanism.

**DISCUSSION**

PPARα, a target for clinically relevant hypolipidemic agents is a lipid-activated nuclear receptor that regulates genes involved in fatty acid metabolism, inflammation, and cell growth. Although it is assumed that the global transcriptional changes occurring upon PP exposure are PPARα-dependent, direct evidence is not available. Here, we show that a prototypical PP, WY-14,643, alters 6.5% of the transcripts in wild-type mouse liver. Of these 815 genes, we found only two genes, the fatty acid ω-hydroxylases Cyp4a10 and Cyp4a14, that were regulated by WY in a PPARα-independent manner. Given that these two genes are also regulated by activators of LXR (25), retinoid X receptor in a PPARα-dependent manner (22), and Nrf2 (14), Cyp4a family members may not be good predictors of PP exposure. The almost complete lack of transcriptional changes in PPARα-null mice after WY exposure was somewhat surprising, given that PPARγ and PPARy are expressed in some cell types in the liver, including Kupffer cells (26), WY is an activator of all three PPAR subtypes (27), and the PP bezafibrate alters gene expression in the liver independently of PPARα (28). It is possible that after WY exposure, transcriptional changes are occurring in nonhepatocyte cell types, but the changes are diluted by the greater number of hepatocyte
transcripts. Our studies demonstrate that PPARα controls the vast majority of transcriptional changes in the mouse liver by a PP.

Prior activation of PPARα suppresses the level of liver damage after subsequent chemical exposure. We showed here that intact livers or primary hepatocytes from PPARα-null mice are more sensitive to damage after exposure to chemicals that induce oxidative stress, including carbon tetrachloride, parquat, and cadmium. Increased sensitivity of PPARα-null mice cannot be attributed to alterations in enzymes that activate these chemicals, since no differences in expression between control wild-type and PPARα-null mice were observed for Cyp2e1 (10), which activates CCl₄. Furthermore, cadmium and parquat do not require bioactivation to induce toxicity. A number of explanations exist for this increased sensitivity to liver damage in PPARα-null mice. PPARα may regulate the timing and extent of hepatocyte repair. In support of this, PPARα is required for optimal repair of the liver after two-thirds partial hepatectomy or chemical-induced damage through regulation of genes involved in cell proliferation (9, 10). Another possibility supported by the transcript profiling studies here is that the hepatocytes from PPARα-null mice have defects in expression of proteins that protect the proteome from chemical damage, including chaperones and proteasomal components (discussed below). Our chemical challenge experiments show that PPARα is required for protection from oxidative stress. The fact that PPARα-null mice exhibit decreased longevity and increased frequency and severity of age-dependent lesions in livers, kidneys, and hearts indicates that PPARα plays an important role in the regulation of genes that determine stress responses and longevity (29).

To identify genes that may be involved in hepatoprotection by PPARα, we compared the gene expression profile of WY with the profiles altered by D3T and heat stress, both of which are known to modify subsequent responses to chemical-induced damage. Three different classes of genes were examined that modulate responses to chemical-induced stress through alteration of the proteome (i.e. protein synthesis, protein folding and stabilization, and protein degradation). WY exposure led to expression changes in mainly protein folding and proteolysis genes with little effect on genes involved in translation. D3T and heat stress gave unique profiles compared with WY; D3T altered genes in all three categories, and heat stress mainly affected a subset of the protein-folding genes. The genes up-regulated by WY included heat shock proteins, chaperonins, and FK506-binding proteins that are involved in different steps in protein folding. Increased expression of Hsp genes by WY is unlikely through alterations in HSF, since WY does not alter HSF1 or HSF2 nuclear localization in rat liver after a 6-day exposure to WY (30). Furthermore, many of the WY-responsive Hsp genes have putative PPREs, and increased expression by WY and heat is additive. Individual overexpression of the WY-induced genes identified here including Hsp25/27 and Hsp70 (11), Hsp86/90 (31–33), and Hsp105/110 (34, 35) protect cells from various stressors including ischemia-reperfusion, heat, and oxidative stress. Future studies will focus on understanding the contribution of these genes to PP-induced hepatoprotection.

A large number of genes encoding components of the proteasome were altered by WY. Both WY and D3T (14, 16) induced the expression of a broad range of proteasomal subunits encompassing both the catalytic core (20 S proteasome) and the ATP-dependent regulatory core (19 S proteasome). Remarkably, all of the subunits of the 20 S proteasome (seven genes in each of the Psma and Psmb families) were induced by WY (p < 0.001), whereas D3T induced 12 of 14 of these genes. Both compounds had little effect on genes involved in ubiquination. The 20 S proteasome can directly degrade oxidatively damaged proteins by recognition of a hydrophobic patch derived from oxidation without assistance from the ubiquination machinery (12). WY and D3T also induced components of the 19 S proteasome, including members of the Psme and Psmd families. The Psme genes are not likely to be regulated directly by WY, since an analysis of 4 kb of the promoter regions revealed putative PPREs in only 6 of the 17 Psme genes examined under search conditions in which PPREs in known PPARα-regulated genes were routinely identified (data not shown). Furthermore, Nrf2 is not induced in WY-induced changes in these genes, since we observed induction in Nrf2-null mice. Induction of Psm genes expression may be an adaptation to decreases in the levels of functional proteasomes. Treatment of cells in vitro with proteasome inhibitors increased the expression of a broad range of subunits of the proteasome in diverse species (13, 36) even when less than 50% of the total proteasomal activity was inhibited (13). Proteasome inhibition resulted in increased expression of 19 and 20 S components but decreased expression of Psmb8 (13), a pattern similar to that observed with WY. Since direct oxidative modification of the catalytic core subunits of the proteasome inhibits their activities (37), WY may be increasing the level of oxidized proteins that inhibit the proteasome, triggering compensatory increases in Psm genes independent of Nrf2 regulation. Given that PPARα activation is down-regulated by the proteasome (38), increases in proteasome activity may be one mechanism to dampen PPARα-dependent gene expression.

In summary, we used transcript profiling to show that PPARα not only determines the vast majority of gene expression changes after exposure to WY but regulates the expression of two classes of genes that may be responsible for protection from chemical-induced oxidative stress: the chaperone genes involved in protein folding and genes involved in proteasomal degradation of damaged proteins. Induction of these potentially protective pathways may provide efficient means for cells to survive conditions of stress from environmental pollutants that contribute to chronic diseases. Induction of these pathways through pharmacological means provides opportunities for protection in a number of settings in which there is induction of oxidative stress, oxidative damage to proteins, and increased occurrence of disease.

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