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HIGHLIGHTS

- CAR activation stimulates a massive liver transcriptional response
- Target genes control drug detoxification, general metabolism, and liver growth
- CAR stimulates genes through coactivation and histone acetylation
- CAR inhibits genes when it shares their enhancers with other nuclear receptors

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Binding of Drug-Activated CAR/Nr1i3 Alters Metabolic Regulation in the Liver

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SUMMARY

The constitutive androstane receptor (CAR/Nr1i3) regulates detoxification of drugs and other xenobiotics by the liver. Binding of these compounds, activating ligands, causes CAR to translocate to the nucleus and stimulate genes of detoxification. However, CAR activation also changes metabolism and induces rapid liver growth. To explain this gene regulation, we characterized the genome-wide early binding of CAR; its binding partner, RXR; and the acetylation that they induced on H4K5. CAR-linked genes showed either stimulation or inhibition and regulated lipid, carbohydrate, and energy metabolism, as well as detoxification. Stimulation of expression increased, but inhibition did not decrease, H4K5ac. Transcriptional inhibition occurred when CAR bound with HNF4a, PPARa, or FXR on the same enhancers. Functional competition among these bound nuclear receptors normally coordinates transcriptional resources as metabolism shifts. However, binding of drug-activated CAR to the same enhancers adds a new competitor that constitutively alters the normal balance of metabolic gene regulation.

INTRODUCTION

The constitutive androstane receptor (CAR/Nr1i3), an important nuclear receptor (NR) transcription factor in hepatocytes, regulates genes that detoxify drugs and xenobiotics. CAR controls an unusually strong transcriptional response that also regulates liver growth and cell proliferation, promotes cancer, and alters glucose metabolism and cholesterol homeostasis (Kobayashi et al., 2015; Tian et al., 2011). CAR-binding activators—drugs and xenobiotics—are agonist ligands that stimulate translocation to the nucleus. Following translocation, CAR heterodimerizes with RXR to bind a characteristic DNA site within transcriptional enhancers (Choi et al., 1997; Wei et al., 2000). This direct repeat 4-binding site (DR4) consists of two 6-bp half sites that bind RXR and CAR, separated by 4 bp. The so-called phenobarbital response element (PBRE) was the first CAR site to be characterized and is −2.3 kb from the transcription start of Cyp2B10, a gene with exceptional induction by CAR activators (Kawamoto et al., 1999). The halogenated hydrocarbon TCPOBOP (1,4-bis[2-(3,5)- dichloropyridyloxy] benzene), a specific ligand for mouse CAR, induces transcription of characteristic target genes from very low basal levels (Columbano et al., 2005; Locker et al., 2003; Tzameli et al., 2000). Moreover, the dramatic growth response to a single dose doubles the liver mass by 18 hr (Tian et al., 2011).

Several close relatives of CAR are important hepatocyte regulators with similar binding sites: homodimeric HNF4a and RXR heterodimeric partners PPARa, FXR, LXRa/β, PXR, and THRa/β. HNF4a is the central regulator of hepatocyte phenotype (Fang et al., 2012). CAR and the other RXR partners control metabolic processes within this phenotype. PPARα regulates genes of fatty acid oxidation and transport following activation by ligands such as unsaturated fatty acids, eicosanoids, and clofibrate drugs (Rakshandehroo et al., 2007). FXR, activated by bile acids, controls cholesterol catabolism by regulating genes of bile acid transporters, apolipoproteins, and carbohydrate and amino acid metabolism (Kim and Moore, 2017). LXR—activated by oxidized cholesterol derivatives—stimulates genes of fatty acid and triglyceride synthesis and cholesterol conversion to bile acids (Boergesen et al., 2012). PXR, like CAR, is a drug and xenobiotic receptor. Ligands for CAR and PXR are generally distinct but their gene regulation overlaps (Cui and Klaassen, 2016). The thyroid hormone receptor (THR) regulates liver genes of lipid, fatty acid, and steroid metabolism (Grontved et al., 2015).

All these NRs activate transcription via their ligand-binding domains (LBDs), which directly bind p160 coactivators. As part of transcriptional activation, the p160s acetylate histones 3 and 4 at several sites, particularly histone 3 lysine 9 (H3K9) and H4K5 (Li et al., 2003; Spencer et al., 1997). In addition, the p160s bind...
another coactivator, CBP, that acetylates H3K27 (Pasini et al., 2010; Tie et al., 2009). Without activating ligands, most NRs can also repress transcription through recruitment of corepressors that deacetylate histones. However, activated NRs stimulate some genes, whereas they inhibit others. This alternate type of inhibition has an important effect on phenotype, but the mechanism is poorly understood.

CAR dimerizes with RXR and binds to DNA like other NRs, but distinctive features simplify its effects and make CAR a valuable tool for examining transcriptional regulation in vivo. (1) Most NRs modulate activation and repression through their LBDs, but the CAR LBD has an unusual conformation that prevents binding of corepressors. CAR is thus a pure transcriptional activator (Suino et al., 2004). (2) CAR resides in the cytoplasm unless ligand or signal transduction activates nuclear transport (Kawamoto et al., 1999). CAR-initiated transcription depends on this translocation. Hence, basal activity is very low but activation of target genes is rapid. (3) CAR lacks an N-terminal activation domain. Such domains provide a second activation mechanism for other NRs.

In this article, we present the chromatin immunoprecipitation sequencing (ChIP-seq) characterization of CAR, with parallel genome-wide characterization of RXR binding, chromatin H4K5 acetylation, and DNaseI hypersensitive sites (DHS). To correlate these dynamic modifications of chromatin with gene expression, we defined the hepatocyte transcriptome, its response to CAR activation, and the association of specific CAR binding sites to regulated genes. The analyses showed distinctive differences between gene stimulation and inhibition by CAR and suggested that inhibition was related to binding of a second NR. We therefore characterized HNF4α binding and its relationship to CAR, followed by analysis of RXR dimeric partners from published datasets. The following study of CAR thus reveals a competitive antagonism among activating NRs that controls the dynamic balance of liver metabolic regulation.

**RESULTS**

**Validation of CAR ChIP-Seq via Candidate Gene Analysis**

CAR activation in the liver transcriptionally regulates genes of detoxification and metabolism while stimulating growth and cell proliferation. The transcriptional responses could reflect direct regulation by CAR or regulation by CAR-induced factors. The responses could also be consequences of general processes like cell proliferation or of indirect mechanisms like depletion of coregulators.

To discriminate direct regulation, we carried out ChIP-seq analysis of CAR binding in liver 3 hr after treatment with TCPOBOP (Figures 1 and S1). The responses are sexually dimorphic, so analysis was simplified by an exclusive focus on young mature female mice without dietary manipulation (Ledda-Columbano et al., 2003; Waxman et al., 1985). CAR antibodies have been problematic, but our prior studies characterized a CAR-binding antibody suitable for conventional ChIP (Tian et al., 2011), and we established highly specific ChIP-seq conditions with this antibody (see Transparent Methods). The specificity is exemplified by the demonstration of 3 CAR-binding enhancers in the region upstream of Cyp2b10 (Figures 1A and 1B), including the PBRE within CAR peak 3 (Kawamoto et al., 1999). The binding coincides with strong transcriptional stimulation (Figure 1C).

CAR detection was complemented by analysis of RXRα, promoter marker H3K4Me3, DNaseI hypersensitivity (DHS), enhancer marker H4K5Ac, and RNA sequencing (RNA-seq). The enhancer marker H4K5Ac was chosen because it directly reflects transcriptional activation by NR (Li et al., 2003; Spencer et al., 1997) and we validated its application by comparing with more common markers of enhancer activation, H3K9Ac and H3K27Ac (Figure S2). For all 3, CAR bound to “valleys” within a field of acetylation. H4K5Ac provided the strongest enhancer detection, the highest discrimination between promoters and enhancers, and greatest changes following TCPOBOP treatment. The relative sensitivity of H4K5Ac detection is exemplified by the changes surrounding CAR enhancer peak 1 (Figure S2B). Because it marked the largest number of enhancer regions, H4K5Ac also provided valuable discrimination of specific and non-specific binding peaks.

Many studies have used DHS to mark enhancers and their activation. However, most enhancer regions of CAR-regulated genes showed only moderate levels of DHS, which was much more prominent at promoters of unrelated genes (Figure S2). Compilation of sequence tags around CAR showed an almost identical distribution of RXR binding in the center of DHS regions. CAR, RXR, and DHS were surrounded by regions of H4K5Ac, which, however, was centrally depleted (Figure 1D). Because of their sensitivity and
complementary relationships, we integrated analysis of NR and H4K5Ac. DHS was analyzed separately, and an additional chromatin modification, H3K4Me3, was used only as a marker of active promoters.

TCPOBOP treatment strongly stimulated CAR binding of the PBRE (peak 3) and two novel enhancers (peaks 1 and 2) near the Cyp2b10 promoter (Figures 1A and 1B). RXR binding and acetylation in control liver indicated that these enhancers were active before CAR binding. Treatment also stimulated transcription of an upstream gene (Cyp2b10us, with 2 spliced isoforms), 5.2 kb from Cyp2b10 (cloned by RACE; GenBank: MF399062, MF399063). Cyp2b10us is a long non-coding RNA without significant open reading frames and...
Figure 2. Characterization of CAR Peaks

(A) The CAR peak set. The plot discriminates H4K5Ac + RXR:CAR (red), CAR-only (black), and peaks excluded because the stimulation ratio was <1.5 (gray).
(B) The RXR peak set. The plot discriminates H4K5Ac + RXR:CAR (red) and other RXR peaks (blue).
(C) The H4K5Ac narrow peak set. The plot discriminates overlapping—CAR + RXR (red), CAR only (black), or RXR only (purple)—and non-overlapping (blue) subsets.
(D) Overlap of CAR, RXR, and H4K5Ac peak sets.
(E) MEME-ChIP analysis showing prominent motifs calculated de novo from peak subsets.
(F) Distribution of CAR DR4 and CEBP motifs on RXR:CAR and CAR-only peak subsets. Control analysis used random sequences matched for length and chromosome location.
lacks homology with the Cyp2b10 transcription unit. Of note, the induction of Cyp2b10us demonstrates that CAR-binding enhancers link to regulation of multiple genes. Two closely related P450 genes, Cyp2b13 and Cyp2b9, are 164 and 276 kb, respectively, from Cyp2b10. Each gene had 2 CAR peaks near the promoter, one with and one without RXR. CAR strongly induced Cyp2b10 and Cyp2b10us, whereas Cyp2b13 and Cyp2b9 were both downregulated but with different time courses (Figure 1C). Induced CAR binding therefore associates with both increased and decreased transcription from different target genes.

Gadd45b, another strongly induced candidate gene (Tian et al., 2011), associated with more distant CAR-binding enhancers within adjacent Gng7, 29–60 kb from the Gadd45b promoter (Figure S1A). In addition to strong CAR-binding peaks, TCPOBOP also induced weak but significant CAR binding to other RXR sites within Gng7, a change noted throughout the genome. Upregulation of another candidate target, Myc, associated with induced CAR peaks at +343 and +361 kb (Figure S1B), whereas the intervening gene Pvt1 showed downregulation. Direct CAR regulation of Myc is possible, since even more distant enhancers control this gene (Uslu et al., 2014) and other regulated genes are far more distant from the induced CAR peaks. Because of the Myc relationship, we used 400 kb as the cutoff for linking CAR peaks to regulated genes.

Characterization of a Specific CAR Peak Set
Candidate analysis detected induced CAR binding near numerous regulated genes and validated the ChIP-seq analysis. We therefore compiled a genome-wide CAR peak set (Feng et al., 2012). Although significant peaks associated with virtually all known CAR targets, they were overshadowed by thousands of much stronger peaks that did not link to regulated genes. These latter peaks were restricted to a small fraction of the genome where similar peaks were detected with control antibodies. We therefore defined 624 genomic segments that could not be analyzed by ChIP-seq (see Transparent Methods and Table S1). These segments—totaling 17.4 mb, 0.7% of the mm9 genome including 62 genes (Table S2)—were filtered before analysis of the remaining 2,553 mb.

Analysis of the remaining genome with MACS2 software (Feng et al., 2012) generated a set of 37,000 CAR peaks with p value < 0.001 (Figures 2A and S3A). Such large peak sets are common, but the number of peaks presents a significant barrier to correlation with specific gene regulation, so secondary cutoffs were derived from binding strength. Elimination of the bottom 2% of the range of peak strength reduced the peak number to 5,400, whereas elimination of 3% left only 3,500 peaks. RXR (9,970 peaks) and H4K5Ac (54,727 peaks) sets (Figures 2B, 2C, S3B, and S3C) were similarly restricted to the top 97% of the range of binding strength. The ratio of top 97% to top 98% peaks was 0.65 for CAR and 0.73 for RXR. The CAR ratio is a little smaller but does not extrapolate to a big difference between expected and observed binding sites.

True CAR peaks have another defining property, binding induced by TCPOBOP treatment. A cutoff ratio of 1.5 (treated/control) excluded nonstimulated peaks, and further reduced the peak set to ~3,000. As a final discriminator, overlap with H4K5Ac-positive regions selected ~2,500 peaks within regions of active transcriptional regulation (Figure 2D).

CAR peaks fell into 2 classes, RXR-CAR and CAR-only peaks. De novo analysis with MEME-ChIP software (Bailey et al., 2015) showed that the RXR-CAR and CAR-only sites were different (Figure 2E). The complete RXR-CAR peak set generated an NR-like CAR half-site motif, but a smaller set—263 peaks that represented the top 90% of the binding strength range—generated a complete CAR-DR4 motif in which the second repeat was nearly identical to the CAR half-site motif. Moreover, an RXR half-site motif, derived from RXR-only peaks, was similar to the first repeat of the DR4 motif. Scanning with the FIMO program (Bailey et al., 2015) then detected matches (p < 0.001) to the CAR DR4 motif in 1,936 of the RXR-CAR peaks, averaging 1.7 motifs per peak.
Competition for limiting RXR is often invoked as a mechanism for transcriptional downregulation, but the net 5% reduction induced CAR bound predominantly to previously active enhancers.

MEME-ChIP analysis of the CAR-only peak set did not derive an NR half-site but instead generated a CEBP motif (Figure 2E). The distribution of CEBP motifs showed central localization in both RXR:CAR and CAR-only sets with a much more significant correlation to the latter (Figure 2F). In contrast, DR4 motifs showed central localization only in RXR:CAR peaks. MEME analysis also derived FOXA motifs from RXR:CAR peaks (E = 1.9e-10), but positional analysis did not show the central localization that would indicate a close association with CAR (not illustrated).

We then compared CAR, RXR, and H4K5Ac in 3 peak sets: RXR:CAR, CAR-only, and RXR-only peaks (Figure 2G). Stimulation of CAR binding was clear in both RXR:CAR and CAR-only sets. Binding of RXR to RXR:CAR sites increased only 5%, confirming that RXR previously bound as a heterodimer to different partners, or as a homodimer, before treatment. Moreover, the moderate increase of H4K5Ac indicated that induced CAR bound predominantly to previously active enhancers.

Competition for limiting RXR is often invoked as a mechanism for transcriptional downregulation, but the binding of RXR to non-CAR sites was unchanged, ruling out this mechanism. However, the net 5% reduction of H4K5Ac on RXR-only sites was moderate but highly significant. This finding suggested an alternate inhibitory mechanism in which new CAR binding competed for H4K5-acetylating cofactors.

**Relating CAR Binding to Gene Expression**

The next studies related the 2,100 RXR-CAR and 400 CAR-only peaks to TCPOBOP-regulated genes. From the 24,000 unique genes in the mm9 Refseq annotation, RNA-seq libraries characterized a 12,000-gene hepatocyte transcriptome and sequential changes through the beginning of S phase at 24 hr (Figure 3A, Table S3). We began by correlating CAR binding with gene expression at 3 hr and found this time interval adequate to study most upregulation, but not downregulation. The variety of sequential regulatory patterns made the problem complex because no single time point was adequate to compare global gene regulation with CAR binding. Separate analysis of multiple time points, however, would have required a prohibitive number of replicate RNA-seq libraries and divided the peaks into small correlations. We therefore used the entire time series to determine the average expression over 24 hr, a normalized hourly value calculated from expression at 0, 3, 6, 12, 18, and 24 hr after TCPOBOP treatment, and used these inclusive 24-hr average values for most correlations.

The analysis exploited the quantitative features of RNA-seq to integrate global changes in the transcriptome with individual genes and pathways. Total transcriptional changes were summed from the individual transcript changes in Table S3 (Figure 3B). A comparison of stimulation/inhibition ratios demonstrated that net 24-hr average transcriptional changes of RNA mass (reads/gene) were balanced when cut off at a 1.2-fold ratio, suggesting a constant level of transcriptional elongation. In contrast, transcript number (reads/gene/kb) showed a 5% net increase of transcriptional initiation that presumably reflected the synthetic requirements of massive liver growth. With this moderate 1.2-fold cutoff, 97% of CAR peaks linked to regulated genes. In contrast, a 2-fold cutoff restricted analysis to the most prominent gene regulations associated with CAR, but linked only 37% of the binding peaks. A 1.2-fold change in gene expression may seem to be a small effect, but can actually reflect a very large increase in transcriptional initiation if the gene is strongly expressed (see Table S3). Moreover, changes of this magnitude for serum proteins, the high-abundance products of the liver transcriptome, have important biological effects.

The vast majority of CAR peaks were in distant regulatory elements within 400 kb of regulated genes (Figures 3C). The median peak-promoter distances for up- and downregulated genes were similar, 34 and
40 kb, respectively. However, for 2-fold regulation, median peak locations were significantly closer to up-regulated (18 kb) and farther from downregulated genes (50 kb).

To assess the biological significance of CAR peak-associated gene expression, functional enrichment analysis (Heinz et al., 2010) compared gene number and statistical significance to ~5,000 profiles (Figure 3D). We also quantified the changes in transcript number for each response. The expected correlations of up-regulated genes to xenobiotic and drug metabolism were clear and significant. Surprisingly, the correlations to lipid metabolism, the insulin-responsive IRS signal transduction pathway, and mitochondrial genes had higher statistical significance and accounted for larger changes in net transcription. In addition, down-regulation dominated the nonxenobiotic responses. CAR induction therefore significantly reprioritized metabolic gene expression in addition to stimulating xenobiotic metabolism.

**Functional Correlation of CAR Binding with Gene Regulation**

Correlation by distance associated CAR-binding elements with 6,400 regulated genes. To examine mechanisms, however, we simplified the relationships to the two regulated genes closest to each CAR-binding peak, a set of ~2,500 genes (Figure 4A, Table S4), which accounted for ~60% of the total transcriptional change. Significantly, both closer and farther genes in the pairs contributed substantial regulation (Figure 4B).

In early studies with the full RXR:CAR peak set, correlation of chromatin changes with gene expression gave biphasic curves, an upward region of positive correlation for increased expression and a flat region of noncorrelation for decreased expression. To resolve these discordant effects, we defined 4 peak subsets with unambiguous relationships to up- or downregulation (Figure 4C). The subsets consisted of peaks associated only with a single regulated gene within 400 kb (single up, n = 94; single down, n = 179) or with 2 genes within 400 kb that had parallel regulation (dual up, n = 282; dual down, n = 572). The remaining peaks had an ambiguous relationship to gene expression, since they associated with oppositely regulated genes (dual opposite, defined by relative distance of the regulated genes as up down, n = 257; or down up, n = 214). The inclusive subsets together comprised 80% of all induced RXR:CAR peaks. The main reason for exclusion was when one of the linked genes only showed a transient expression change at 3 hr.

Analysis of H4K5Ac in subsets revealed a clear difference between up- and downregulation (Figure 4C). The former had increased acetylation, the expected effect of an activating NR. The latter, however, showed little change, not the histone deacetylation expected for true repression. The dual opposite pairs had intermediate changes dominated by regulation of the closer gene. A general comparison showed highly significant differences between up- and downregulation across the different subsets (Figure 4D).

Detailed characterization of peak subsets and linked gene expression is presented in Figure S4. Transcript number changes, CAR pileup, and CAR-binding stimulation demonstrated overall similarity of the subsets, which validated their use in our analysis. Subset expression ratios also confirmed differences between up- and downregulation (Figure S4A). Upregulation was faster and sustained, since 3- and 24-hr average median expression ratios were similar. Downregulation was delayed—median expression ratios were 0.91–0.94 at 3 hr and 0.66–0.68 in the 24-hr average. The 3-hr ratio medians were of particular interest, because even though the effects were small, they still confirmed that downregulation started soon after CAR binding. The delay most likely represents slow turnover of mRNA following inhibition by CAR. Subset comparisons also confirmed that downregulation generally occurred over a greater distance than upregulation (Figures 3C and S4E).

Parallel analysis of DHS showed differential changes in the CAR-binding peak center (Figure S5). Upregulation caused slight decrease, not the expected increase in DHS, whereas peaks of downregulated genes showed a greater but still moderate decrease. The decreases probably reflect local remodeling by altered binding of transcription factors and cofactors. The consistent differences in H4K5Ac and DHS changes confirm that up- and downregulation occur by different mechanisms rather than reciprocal changes in a single process, but neither change explains the cause of downregulation.

**Regulation by CAR-Only Sites**

The use of peak subsets also enabled characterization of gene regulation by CAR-only sites (Figure S6). Many CAR-only peaks regulated the same genes as RXR:CAR peaks (Table S4). However, we established...
Figure 4. Refinement of the Relationship of CAR Peaks to Regulated Genes

(A) Simplification of transcriptome analysis. All groups represent expression of 24,000 mm9 transcripts with unique Refseq gene annotations. Enriched, hepatocyte-enriched compared to whole liver, >0.1 transcript per cell; Regulated, >20% 24-hr average change after treatment with TCPOBOP; CAR < 400 kb, regulated genes within 400 kb of an induced CAR peak; 2 genes, the two closest regulated genes to each CAR peak.

(B) Contributions of the 2 closest genes to regulation. Number fractions were calculated as in Figure 3B.

(C) Histogram analysis of H4K5Ac in defined peak gene subsets. H4K5Ac increased in subsets associated with upregulation but showed little change in genes associated with downregulation. r, ratio of treated/control peak summits; p, paired t test comparing control and treated H4K5Ac values of individual peaks, as in Figure 2G.

(D) H4K5Ac correlations among peak sets. Sets of individual peak ratios were compared using unpaired t tests.
two purely noncanonical subsets, 58 stimulatory and 112 inhibitory CAR-only peaks that were exclusively linked to 284 genes. Downregulation predominated for these moderately expressed genes while ontology analysis related them to protein modification processes and ion binding, a distinctive phenotypic contribution.

**Downregulation via Antagonism of HNF4α**

Homodimeric HNF4α shares many gene targets with CAR (Tirona et al., 2003), so we analyzed H4K5Ac-positive HNF4α binding and defined a set of ~10,000 peaks (Figures 5A, S7A, and S7B). The HNF4α peak characterization used restrictions similar to the CAR and RXR peak sets. Of the RXR:CAR peaks 70% coincided with HNF4α peaks (Figures 5A and 5B, Table S4). Regulated genes linked to HNF4α binding showed minimal change with treatment. HNF4α binding was strongest over the sites that were overlapping or adjacent, although statistical significance was limited by small peak sets. Peak maxima were calculated from histogram analysis. The analyses compared 3 sets: All (in subsets), Dual up down, and Dual down up peaks. HNF4α + represents all positive peaks, then subdivided into <12-, 12- to 26-, and >26-bp groups. HNF4α - represents all negative peaks. Statistical analysis compared HNF4α levels for individual HNF4α + groups with HNF4α binding of all positive peaks (the 1,009 peak set on the far left) using unpaired t tests; n, the number of peaks in each group; *p < 0.05, **p < 0.01.

(H) Relationship of HNF4α to the ontology of CAR-regulated lipid metabolism. The global lipid gene profile from Figure 3I was elaborated with separate profiles that represent different aspects of lipid metabolism. The plot quantifies TCPOBOP-induced transcriptional changes linked to HNF4α + and HNF4α - CAR peaks. p values for detection of genes within individual profiles: **p <0.01, ***p <0.001, ****p <0.0001, *****p <0.000001.

The opposing regulation of dual opposite gene pairs suggested a competition that transferred transcriptional initiation and HNF4α had a selective effect on the linked RXR:CAR peaks in these subsets. HNF4α + peaks showed a strong increase in H4K5Ac, whereas the level was unchanged in HNF4α - peaks (Figure 5E). This effect was unexpectedly limited to dual opposite gene pairs since the increased H4K5Ac of single up and dual up peaks was independent of HNF4α binding. Similarly, the H4K5Ac levels of single down and dual down peaks did not change with or without HNF4α (Figure S7C).

One possible explanation for the differential acetylation of HNF4α-positive and HNF4α-negative sites was binding competition with RXR:CAR. To investigate such competition, distances between CAR and HNF4α peak centers were classified to define overlapping (<12 bp center-center distance), adjacent (12-26 bp), or separate (>26 bp) binding sites (Figure 5F). Surprisingly, HNF4α binding was unchanged by TCPOBOP treatment (Figure 5G). The data instead showed the strongest HNF4α binding at overlapping and adjacent sites. The opposing effects of CAR and HNF4α on gene expression thus does not arise from direct binding competition at common sites.

The opposing effects on gene expression of CAR and HNF4α on dual opposite gene pairs had strong phenotypic consequences. These are exemplified by ontologic characterization of separate pathways (Figure 5H) within the general profile cellular lipid metabolic processes (Figure 3D). CAR binding at HNF4α - peaks associated with decreased synthesis of some lipids, fatty acids, and lipoproteins. In contrast, CAR binding at HNF4α + peaks associated with increased fat digestion and absorption, and synthesis of other lipids and lipoproteins.

**Common Regulation by a Network of RXR Partners**

The antagonism of HNF4α suggested that CAR could have similar effects on other NRs. To evaluate their expression, the entire set of hepatocyte NR was quantified from the RNA-seq libraries along
with selected coregulators (Figures 6A and 6B). Strong effects on Ppara and the coregulator Ppargc1b were notable, each with downregulation of about 60% that was associated with nearby RXR-CAR binding.

Since PPAR, FXR, and LXR are all activated by endogenous ligands, we compiled peak sets of the basal state from published datasets (Boergesen et al., 2012; Thomas et al., 2010). ChIP-seq data was also obtained from a THR study that compared hypothyroid propylthiouracil-treated mice with and without T3 for 5 days (Grontved et al., 2015) and from a PXR study that characterized livers 12 hr after a single dose of pregnenolone 16α-carbonitrile treatment, and THR after T3-treatment. Filtering removed peaks with p > 0.001, the bottom 3% of the range of binding strength, or absence of H4K5Ac, suggesting heterogeneity due to unidentified binding partners. Non-overlapping PXR peaks only generated a half-site motif closely related to CAR. For each NR motif, the class of binding site is shown in bold type and the E value in parentheses.

Throughout the genome, the NR peaks frequently overlapped. For example, the 80-kb region upstream of ChREBP/Mlxipl—an important transcription factor that integrates glycolysis and lipogenesis—displayed CAR, HNF4α, RXR, FXR, LXR, PPAR, PXR, and THR peaks, overlapping in different combinations (Figure 6C).

Overlap of these RXR partners with CAR was frequent (Figure 6D, Table S4). PPARx formed the largest set of RXR dimeric peaks (5,358) and also had the largest overlap fraction with RXR:CAR peaks, 65%. Other overlap fractions were FXR, 56%; LXR, 39%; PXR, 39%; and THR, 9%. MEME analysis of overlapping subsets yielded only NR half-site motifs (not illustrated). In contrast, MEME generated characteristic dimeric motifs for non-overlapping CAR, PPAR, FXR, LXR, THR, and HNF4α sites that validated the peak compilations (Figure 6E). PXR and RXR peaks were exceptions. Their non-overlapping sets were probably heterogeneous since they generated only half-site motifs. Compared to the 17% of CAR-only peaks, the PPAR, FXR, LXR, PXR, and THR analyses demonstrated more RXR− peaks, 28%–50%. These RXR− peaks—NR-specific noncanonical binding or technical differences among diverse datasets—were filtered before comparative analysis. The distribution of binding peaks, representation of known binding sites, and linkage to regulated genes all indicated that the peak sets were adequate for correlation of their binding in liver.

**Phenotypic Effects of Coregulation by CAR and Other NR**

Since acetylation of the dual opposite peaks provided a sensitive assessment of antagonism by HNF4α, we used these peak sets to analyze interactions with PPAR, FXR, LXR, PXR, and THR (Figure 7A). Peaks with CAR and a second NR always had higher basal acetylation, presumably reflecting their activation by both. However, sites that lacked PPAR or FXR—like those that lacked HNF4α—showed lower basal acetylation and stronger stimulation of acetylation by TCPOBOP. These acetylation states discriminated antagonism of CAR and the other NR but did not explain the antagonism because high acetylation was maintained. For LXR and THR, treatment gave nearly equivalent stimulation of positive and negative sites, a neutral relationship. PXR distinctively showed a cooperative relationship with CAR, since TCPOBOP stimulated acetylation of PXR-positive more than PXR-negative peaks.

Further analysis resolved the effects of a second cobinding NR from those of HNF4α, which bound to many of the same peaks (Figure 7B). The inhibition of acetylation by PPARα and FXR was even greater than the inhibition by HNF4α, consistent with strong antagonism of CAR. Again, LXR and THR were neutral, and PXR
showed cooperation with CAR. Comparison of peak centers showed that some cobinding NR directly overlapped with CAR (Figure 7C). However, NR position within enhancers showed little relationship to stimulation of H4K5Ac (not illustrated).

An assessment of phenotypic effects then correlated CAR-linked genes with published expression profiles of PPAR, FXR, LXR, PXR, and THR (Boergesen et al., 2012; Grøntved et al., 2015; Oshida et al., 2015; Rakhshandehroo et al., 2007; Zhan et al., 2014). Individual CAR-linked genes in these profiles showed either concordant or discordant regulation by the second NR (Table S5, Figure 7D). In the two largest discordant sets, CAR shared 225 and 92 enhancers with PPAR and FXR, respectively. In contrast, CAR inhibition of genes stimulated by PXR or THR linked to separate enhancers. Phenotypically, the antagonistic binding of CAR suggested important metabolic consequences, inhibition of lipid metabolism genes stimulated
DISCUSSION

Modeling CAR Regulation

The studies in this article correlated genome-wide CAR binding in the liver with changes in the hepatocyte transcriptome. In the absence of specific perturbation of individual sites and genes, such analysis can only be correlative. The analysis used unbiased strategies to enrich the quality of these correlations. The article also provides resources with full data on each CAR peak and regulated gene (Tables S3 and S4).

Peak sets were refined by eliminating those that represented library artifacts, low statistical significance, weak binding, and lack of association with active chromatin. The most important refinement was the elimination of weak binding sites despite the statistical significance of most of these detections. Elimination was justified by their weak interaction and poor correlation with binding-site motif analysis and gene regulation. Statistical restriction, even to very low p values, did not eliminate most weak sites (see Figures S3A–S3C and S7A). The final sets of 2,500 CAR peaks (2,100 RXR:CAR peaks), 9,100 RXR peaks, and 10,000 HNF4α peaks are highly representative of observed hepatocyte gene regulation.

Transcriptome correlation used comparable refinement, first by limiting analysis to hepatocyte-expressed CAR-linked genes regulated by TCPOBOP treatment. More specialized restrictions were necessary to enrich specific regulatory relationships: two genes closest to each induced CAR-binding peak (~2,500 genes) and CAR peak subsets with defined linkage to either up or down 24-hr average regulation (~1,600 peaks). Despite the relatively small size of these sets, they still related 80% of RXR:CAR peaks to 70% of the CAR-linked change in transcriptional initiation. The aggregate measurement of H4K5Ac in these sets showed different effects if peaks were linked to gene pairs with up, down, or opposite regulation. The separate H4K5Ac relationships validate the unbiased selection criteria and indicate a high degree of homogeneous enrichment.

The analyses led to a specific view of gene regulation in mature liver. CAR did not activate a program of differentiation or repression. Changes in gene expression, even the strongest, linked to sites that already bound RXR within hypersensitive regions surrounded by acetylated chromatin. CAR therefore modulated active genes within the mature liver phenotype. Modulation of active genes also characterized liver regulation by other RXR partners.

Properties of CAR Regulation

The minimal CAR binding in control liver presumably reflected a low level of endogenous or dietary ligands. Nevertheless, the vast majority of heterodimeric sites (92%) were occupied by RXR before TCPOBOP treatment, suggesting prior regulation by other NR:RXR pairs. In addition, many of the 7,000 “CAR-negative” RXR peaks actually showed weak CAR binding that reflects the moderate differences in specificity of NR-binding sites.

Most CAR peaks corresponded to distant enhancers, typical of gene regulation by NR. Use of a 400-kb distance cutoff led to inclusion of a few interesting regulations, but few regulatory peaks were very distant and quantifications using a 150-kb distance cutoff were virtually the same (not shown).

The aggregate analysis of large peak sets in some ways contradicts the examination of exceptional candidate genes, which may have outlying properties. To examine their special regulatory mechanisms, we investigated a variety of outlying subsets for correlation with other NR binding, chromatin acetylation, binding motifs, gene expression, and ontology. The outlier CAR peak sets had basal liver detection (245 peaks), unusually strong binding (263 peaks), de novo binding of RXR and CAR (99 peaks), or a >2-fold change in acetylation, up (48 peaks) or down (19 peaks). The strongly binding peaks had a moderate positive correlation with the strength of gene induction after TCPOBOP treatment and generated the clearest binding motif, but none of the other outlying subsets showed a significant correlation with differences in a second property or with ontology profiles. Moreover, genes regulated by these outliers often associated with additional peaks that were more typical.
Transcriptional Antagonism of CAR with Other NR

Most CAR peaks overlapped with HNF4α or other NR. Regardless of this overlap, peaks associated with upregulated genes increased acetylation. CAR activation also induced downregulation, but the mechanism was not clear. CAR peaks associated with oppositely regulated genes provided a valuable resource for examining this mechanism since opposing regulation suggested transfer rather than loss of activation. In these *dual opposite* CAR peaks, increased acetylation inversely correlated with HNF4α (or other NR) binding. Thus, downregulation could be mediated by competition between HNF4α and CAR. Binding site occupancy, however, was not decreased even when sites directly overlapped. Instead, overlapping binding sites had the strongest binding of both CAR and HNF4α. The likely explanation for the lack of binding competition is site sharing, which can occur if the time of residency is brief. Moreover, binding of one NR at a shared site can increase occupancy by a second via assisted loading if the first has modified chromatin to improve accessibility (Voss et al., 2011). Short residency time and assisted loading, however, still do not explain transcriptional downregulation or the apparent competition for expression of nearby genes.

It is important to consider two alternate explanations for overlapping binding. The first is that different NRs might bind in separate cell populations. This explanation is incompatible with the observed interactions of CAR with HNF4α, PPAR, and FXR (Figures 5E, 7A, and 7B). Functional relationships with LXR, PXR, and THR are more ambiguous, but coexpression of these NRs with CAR in the same hepatocytes is well known. The second explanation is that binding of CAR might be indirect. Indeed, indirect CAR binding to CEBP is likely for CAR-only sites, but indirect binding of CAR to bound HNF4α dimers is unlikely. Moreover, the occupied sites bind CAR, HNF4α, and RXR and have canonical dimeric binding motifs compatible for either RXR-CAR or HNF4α.

The NRs of this study (Figure 8A) share domain structure, dimeric binding to similar paired half-sites, and activation through the LBD and its AF2 region. In the typical activation process, the LBD recruits a p160 coactivator that acetylates histones at several sites including H4K5 (Li et al., 2003). The LBD and p160 then recruit CBP and other coregulators to further modify chromatin, link to a distant promoter, and stimulate the formation of a preinitiation complex (Rosenfeld et al., 2006). CAR and HNF4α constitutively mediate this LBD-driven process; PPAR, FXR, LXR, PXR, and THR require an activating ligand; and RXR is usually considered a passive partner (Evans and Mangelsdorf, 2014). Except for PXR (and CAR), however, activating ligands are normally present in the liver.

NRs share a common activation mechanism through the LBD and its coactivators. A second NR on the same enhancer might compete for these coactivators but would then have an equivalent transcriptional effect. Coactivator competition could, however, explain how CAR downregulates unlinked genes, an effect suggested by the reduced acetylation of CAR-negative RXR sites (Figure 2G).

Many NRs have a second activation domain (AF1) at the N terminal. AF1 domains differ and can provide specific mechanisms for individual NR. CAR is the most compact NR in this study, without an N-terminal domain or AF1. The functions of some AF1 regions are uncharacterized, but two other hepatocyte NRs have known special features. TRIB1 and CTNNB1 are AF1-specific cofactors for HNF4α and FXR, respectively (Liu et al., 2015; Soubeyrand et al., 2017). HNF4α also has a distinctive F-domain at the C terminal that modulates the function of AF2 (Sladek et al., 1999).

NR competition, for binding or coactivators, does not explain downregulation or the reciprocal changes of genes linked to the same enhancers. Gene competition, the competition of promoters for local enhancers, provides an alternative mechanism that can explain the behavior of opposite gene pairs (Bulger and Groudine, 1999; Quintero-Cadena and Sternberg, 2016). Several observations are consistent. (1) The great majority of regulated promoters are distant from their CAR-binding enhancers. Moreover, 378 regulated gene pairs—including 225 with opposite regulation—link to single CAR-binding peaks. (2) The opposite regulation of gene pairs suggests that activation shifts from one gene to the other. (3) Downregulating enhancers are farther from the promoters than upregulating enhancers (Figures 3C and 5E), suggesting that promoter distance affects competition.

We therefore hypothesize that an additional NR will alter the competition for local promoters to increase diversion of an enhancer to a second gene. This will downregulate the first and upregulate the second (Figure 8B). The effect on local gene competition can be small or substantial for individual genes and maintains...
a regional balance of expression. The effects result from a shift of activation and modulate an established phenotype without changing it.

As an example, changes in the lipoprotein Apoa1, Apoc3, Apoa4 and Apoa5 gene cluster made large contributions to the transcription fraction. The 4 genes cluster in a 43-kb region of chromosome 9 with 2 CAR peaks—one HNF4α+ and both PPARγ, FXR, and PXR+. In response to TCPOBOP, expression of Apoa1 (1.3 X) and Apoa4 (4.1 X) went up, whereas that of Apoc3 (0.8 X) and Apoa5 (0.8 X) went down.

**Relationship to Other NRs**

The most prominent CAR-NR interaction was with HNF4α, an essential regulator of early liver development and the mature hepatocyte phenotype (Parviz et al., 2003). HNF4α and CAR share many target genes through ~1,400 common enhancers. However, constitutive HNF4α activation does not change in response to physiologic signals or ligands (Yuan et al., 2009). CAR and the other RXR partners therefore mediate dynamic change by competing within, and borrowing transcriptional resources from, the HNF4α-controlled phenotype.

NR-controlled gene competition can account for the reciprocal stimulation and inhibition between the fed and fasted states mediated by FXR and PPARα (Kim and Moore, 2017). These NRs have common binding sites near “more than 1,100 target genes that showed opposite responses much more frequently than concordant responses.” CAR also modulates these responses, since ~1,000 peaks bind PPAR, FXR, and
CAR. CAR-mediated downregulation of targets of IRS1 and IRS2, a profile derived from Irs1<sup>−/−</sup> and Irs2<sup>−/−</sup> mice, highlights its antagonism of FXR and PPAR<sub>x</sub> (Cipriani et al., 2010; Guo et al., 2009; Guerre-Millo et al., 2001). CAR also antagonizes PPAR by a second significant effect, transcriptional downregulation of Ppara.

**Drug Responses Are Different from Ordinary Metabolic Regulation**

A critical property that discriminates CAR from HNF4<sub>a</sub> and other RXR partners is its inactivity in basal liver. The induced CAR response is very strong and thus diverts the normal regulatory network. The combination of metabolic reprioritization with rapid hepatocyte growth and proliferation suggests that the CAR response evolved to deactivate toxic agents and quickly replace the hepatocytes that they kill.

The RXR network normally regulates metabolic genes that respond to cyclic stimuli from feeding and activity. The network coordinates the timing of these responses and balances the utilization of limiting transcriptional resources (Kim and Moore, 2017). Drugs are a special class of xenobiotics, selected because they are relatively nontoxic and have valuable therapeutic properties. CAR-induced proteins like p450 cytochromes inactivate these drugs, but CAR also rebalances the metabolism of lipids and glucose tolerance. Maintaining a significant drug level can therefore apply a continuous perturbation to the cyclic regulatory networks and produce metabolic changes, off-target side effects that are unrelated to the direct action of the inducing drug.

**Limitations of the Study**

This study focused on short-term changes to show how CAR controls liver metabolism. The study was deliberately limited to female mice to simplify the analysis of this sexually dimorphic response. Species differences are also important, since differences among the responses of mouse, rat, and human livers are well known. Moreover, CAR activation in a natural setting may associate with toxic agents that cause liver damage, which will significantly modify the response. These important biological differences must be characterized in future studies, particularly to understand human responses and their application to therapy.

At a mechanistic level, our analysis could only correlate CAR binding at distant enhancers with changes in gene expression because there was no demonstration that modification of specific enhancers altered the activation of putative target genes and there was no analysis of enhancer-promoter contact. The data also suggest that enhancers can interact with each other, a similar kind of linked interaction. Indeed, the relationships among promoters and distant enhancers are frequently ambiguous. Although beyond the scope of this article, more specific characterization of these interactions is an important area for new research.

Another limitation was our use of specific chromatin acetylations to characterize changes induced by CAR binding. The main analysis of H4K5Ac had a strong mechanistic link to NR activation, gave clear quantifiable changes, and provided distinctive correlations with types of gene expression. Nevertheless, the integrated study of H4K5Ac, H3K27Ac, H3K9Ac, DHS, and other chromatin modifications, could provide a detailed view of the progressive enhancer changes that lead to transcriptional activation following NR binding. CAR has rapid and strong induction from a minimal basal state in the liver and therefore provides an ideal system for such studies of natural transcriptional activation in vivo.

**METHODS**

All methods can be found in the accompanying Transparent Methods supplemental file.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Transparent Methods, seven figures, and five tables and can be found with this article online at https://doi.org/10.1016/j.isci.2018.10.018.

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DECLARATION OF INTERESTS
The authors declare no competing interests.

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REFERENCES
Bailey, T.L., Johnson, J., Grant, C.E., and Noble, W.S. (2015). The MEME suite. Nucleic Acids Res. 43, W39–W49.

Boergesen, M., Pedersen, T.A., Gross, B., van Heenings, S.J., Hagenbeek, D., Bindesboll, C., Caron, S., Lalloyer, F., Steffensen, K.R., Nebb, H.I., et al. (2012). Genome-wide profiling of liver X receptor, retinoid X receptor, and peroxisome proliferator-activated receptor alpha in mouse liver reveals extensive sharing of binding sites. Mol. Cell. Biol. 32, 852–867.

Bulger, M., and Groudine, M. (1999). Looping versus linking: toward a model for long-distance gene activation. Genes Dev. 13, 2465–2477.

Choi, H.S., Chung, M., Izameli, I., Simha, D., Lee, Y.K., Seol, W., and Moore, D.D. (1997). Differential transactivation by two isoforms of the orphan nuclear hormone receptor CAR. J. Biol. Chem. 272, 23565–23571.

Copiani, S., Mencarelli, A., Palladino, G., and Fiorucci, S. (2010). FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (fa/fa) obese rats. J. Lipid Res. 51, 771–784.

Columbano, A., Ledda-Columbano, G.M., Pibiri, M., Cossu, C., Menegazzi, M., Moore, D.D., Huang, W., Tian, J., and Locker, J. (2005). Gadd45beta is induced through a CAR-dependent, TNF-independent pathway in murine liver hyperplasia. Hepatology 42, 1118–1126.

Cui, J.Y., Gunewardena, S.S., Rockwell, C.E., and Claassen, C.D. (2010). ChiPing the cistrome of PXR in mouse liver. Nucleic Acids Res. 38, 7943–7963.

Cui, J.Y., and Claassen, C.D. (2016). RNA-Seq reveals common and unique PXR- and CAR-target gene signatures in the mouse liver transcriptome. Biochim. Biophys. Acta 1859, 1198–1217.

Evans, R.M., and Mangelsdorf, D.J. (2014). Nuclear receptors, RXR, and the big bang. Cell 157, 255–266.

Fang, B., Mane-Padros, D., Bolotin, E., Jiang, T., and Sladek, F.M. (2012). Identification of a binding motif specific to HNF4 by comparative analysis of multiple nuclear receptors. Nucleic Acids Res. 40, 5343–5356.

Feng, J., Liu, T., Qin, B., Zhang, Y., and Liu, X.S. (2012). Identifying ChIP-seq enrichment using MACS. Nat. Protoc. 7, 1728–1740.

Grontved, L., Waterfall, J.J., Kim, D.W., Baek, S., Sung, M.H., Zhao, L., Park, J.W., Nielsen, R., Walker, R.L., Zhu, Y.J., et al. (2015). Transcriptional activation by the thyroid hormone receptor through ligand-dependent receptor recruitment and chromatin remodelling. Nat. Commun. 6, 7048.

Guo, S., Coppins, K.D., Dong, X., Park, S., Cheng, Z., Pocai, A., Rossetti, L., Saajan, M., Farese, R.V., and White, M.F. (2009). The Irs1 branch of the insulin signaling cascade plays a dominant role in hepatic nutrient homeostasis. Mol. Cell. Biol. 29, 5070–5083.

Heinz, S., Bennet, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol. Cell. 38, 576–589.

Kawamoto, T., Sueyoshi, T., Zelko, I., Moore, R., Washburn, K., and Negishi, M. (1999). Phenobarbital-responsive nuclear translocation of the receptor CAR in induction of the CYP2B gene. Mol. Cell. Biol. 19, 6318–6322.

Kim, K.H., and Moore, D.D. (2017). Regulation of liver energy balance by the nuclear receptors farnesoid X receptor and peroxisome proliferator activated receptor alpha. Dig. Dis. 35, 203–209.

Kobayashi, K., Hashimoto, M., Honakoski, P., and Negishi, M. (2015). Regulation of gene expression by CAR: an update. Arch. Toxicol. 89, 1045–1052.

Lajugie, J., and Bouhassira, E.E. (2011). GenPlay, a multipurpose genome analyzer and browser. Bioinformatics 27, 99–112.

Oshida, K., Vasani, N., Thomas, R.S., Applegate, D., Gonzalez, F.J., Alekoumnes, L.M., Klaassen, C.D., and Corton, J.C. (2015). Screening a mouse liver gene expression compendium identifies modulators of the aryl hydrocarbon receptor (AhR). Toxicology 326, 99–112.

Parviz, F., Matullo, C., Garrison, W.D., Savatski, L., Adamson, J.W., Ning, G., Kaestner, K.H., Rossi, J.M., Zaret, K.S., and Duncan, S.A. (2003). Hepatocyte nuclear factor 4alpha controls the development of a hepatic epithelium and liver morphogenesis. Nat. Genet. 34, 292–296.

Pasini, D., Malatesta, M., Jung, H.R., Walfirdsson, J., Willer, A., Olsson, L., Skotte, J., Wutz, A., Porse, B., Jensen, O.N., et al. (2010). Characterization of an antagonistic switch between histone H3 lysine 27 methylation and acetylation in the transcriptional regulation of Polycomb group target genes. Nucleic Acids Res. 38, 4958–4969.

Quintero-Cadena, P., and Sternberg, P.W. (2016). Enhancer sharing promotes neighborhoods of transcriptional regulation across eukaryotes. G3 (Bethesda) 6, 4167–4174.

Rakhshandehroo, M., Sanderson, L.M., Matlaiinen, M., Stienstra, R., Carlberg, C., de Groot, P.J., Muller, M., and Kersten, S. (2007). Comprehensive analysis of PPARalpha-dependent regulation of hepatic lipid...
metabolism by expression profiling. PPAR Res. 2007, 26839.

Rosenfeld, M.G., Lunyak, V.V., and Glass, C.K. (2006). Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. Genes Dev. 20, 1405–1428.

Sladek, F.M., Ruse, M.D., Jr., Nepomuceno, L., Huang, S.M., and Stallcup, M.R. (1999). Modulation of transcriptional activation and coactivator interaction by a splicing variation in the F domain of nuclear receptor hepatocyte nuclear factor 4alpha1. Mol. Cell. Biol. 19, 6509–6522.

Soubeyrand, S., Martinuk, A., and McPherson, R. (2017). TRIB1 is a positive regulator of hepatocyte nuclear factor 4-alpha. Sci. Rep. 7, 5574.

Spencer, T.E., Jenster, G., Burcin, M.M., Allis, C.D., Zhou, J., Mizzen, C.A., McKenna, N.J., Onate, S.A., Tsai, S.Y., Tsai, M.J., et al. (1997). Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389, 194–198.

Suino, K., Peng, L., Reynolds, R., Li, Y., Cha, J.Y., Repa, J.J., Kliever, S.A., and Xu, H.E. (2004). The nuclear xenobiotic receptor CAR: structural determinants of constitutive activation and heterodimerization. Mol. Cell 16, 893–905.

Thomas, A.M., Hart, S.N., Kong, B., Fang, J., Zhong, X.B., and Guo, G.L. (2010). Genome-wide tissue-specific farnesoid X receptor binding in mouse liver and intestine. Hepatology 51, 1410–1419.

Tian, J., Huang, H., Hoffman, B., Liebermann, D.A., Ledda-Columbano, G.M., Columbano, A., and Locker, J. (2011). Gadd45β is an inducible coactivator of transcription that facilitates rapid liver growth in mice. J. Clin. Invest. 121, 4491–4502.

Tie, F., Banerjee, R., Stratton, C.A., Prasad-Sinha, J., Stepanik, V., Zlobin, A., Diaz, M.O., Scacheri, P.C., and Harte, P.J. (2009). CBP-mediated acetylation of histone H3 lysine 27 antagonizes Drosophila Polycist silencing. Development 136, 3131–3141.

Tirona, R.G., Lee, W., Leake, B.F., Lan, L.B., Cline, C.B., Lamba, V., Parviz, F., Duncan, S.A., Iruce, Y., Gonzalez, F.J., et al. (2003). The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. Nat. Med. 9, 220–224.

Tzameli, I., Pissios, P., Schuetz, E.G., and Moore, D.D. (2000). The xenobiotic compound 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene is an agonist ligand for the nuclear receptor CAR. Mol. Cell. Biol. 20, 2951–2958.

Uslu, V.V., Petretich, M., Ruf, S., Langenfeld, K., Fonseca, N.A., Marioni, J.C., and Spitz, F. (2014). Long-range enhancers regulating Myc expression are required for normal facial morphogenesis. Nat. Genet. 46, 753–758.

Voss, T.C., Schiltz, R.L., Sung, M.H., Yen, P.M., Stamatoyannopoulos, J.A., Biddie, S.C., Johnson, T.A., Miranda, T.B., John, S., and Hager, G.L. (2011). Dynamic exchange at regulatory elements during chromatin remodeling underlies assisted loading mechanism. Cell 146, 544–554.

Waxman, D.J., Dannan, G.A., and Guengerich, F.P. (1985). Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoforms. Biochemistry 24, 4409–4417.

Wei, P., Zhang, J., Egan-Hafley, M., Liang, S., and Moore, D.D. (2000). The nuclear receptor CAR mediates specific xenobiotic induction of drug metabolism. Nature 407, 920–923.

Yuan, X., Ta, T.C., Lin, M., Evans, J.R., Dong, Y., Bolotin, E., Sherman, M.A., Forman, B.M., and Sladek, F.M. (2009). Identification of an endogenous ligand bound to a native orphan nuclear receptor. PLoS One 4, e5609.

Zhan, L., Liu, H.X., Fang, Y., Kong, B., He, Y., Zhong, X.B., Fang, J., Wan, Y.J., and Guo, G.L. (2014). Genome-wide binding and transcriptome analysis of human farnesoid X receptor in primary human hepatocytes. PLoS One 9, e105990.
Supplemental Information

Binding of Drug-Activated CAR/Nr1i3
Alters Metabolic Regulation in the Liver

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Figure S1. Distant CAR binding associated with two candidate target genes

(A) Gadd45b and adjacent Gng7. Gadd45b is one of the genes most strongly induced by TCPOBOP The region with the closest CAR peaks is within the adjacent gene, Gng7.

(B) Myc and adjacent Pvt1. Among candidate genes stimulated more than 2-fold by TCPOBOP, Myc had the most distant linked CAR binding enhancers. Nevertheless, these enhancers were found in a region where even more distant Myc enhancers are known.

RNA-seq is displayed with log_{10} and ChIP-seq with linear scales, using GenPlay. Table values are described in Figure 1. Transcript quantifications by RNA-seq were normalized to the values of untreated liver. p values for the time series were calculated by comparison to Ppia mRNA (see Table S3).
Figure S2. Comparison of chromatin markers in a 1.7 mb region around Cyp2b10.

GenPlay visualizations compare promoter-specific H3K4Me3 with acetylated histones H3K9Ac, H3K27Ac, and H4K5Ac, and DNaseI hypersensitivity (DHS), 4 markers that characterize both promoters and enhancers.

A. Overview of a 1.7 Mb region of chromosome 7. The Cyp2b10-Cyp2b13-Cyp2b9 region displayed in Figure 1A is marked. Note that genes in this region have active promoters with discrete H3K4Me3 peaks, also marked by DHS and the 3 acetylations. The acetylations also mark broader enhancer regions. H4K5Ac provides the strongest detection of enhancer regions and the weakest detection of promoter regions. DHS is a much weaker marker of these enhancer regions. All markers are absent from a distal region of unexpressed genes (Nlrp4a, Nlrp9a, Cyp2b23). For comparison, a proximal region containing mostly housekeeping genes (e.g., Hnrnpul1, Ccdc97, Bckdha) shows strong DHS and acetylation peaks overlying the H3K4Me3-positive promoter peaks. The patterns in this region show that the overall DHS analysis was sensitive despite its weak localization to enhancer regions.

B. Expanded view of the region containing 3 CAR-binding enhancers near Cyp2b10. The tracks show the differences calculated by subtracting control from TCP0BOP-treated levels and were amplified to optimally display the subtracted differences. The tracks in panel A more accurately reflect the relative detection sensitivity of H4K5Ac, H3K27Ac, H3K9Ac, and DHS. Note that only H4K5Ac shows strong differences associated with induced CAR peak 1. TCP, TCP0BOP treatment for 3 hr; CTL, untreated control.
Figure S3. Restriction of CAR, RXR, and H4K5Ac peak sets

(A) CAR. CAR ChIP-seq libraries were compiled with MACS2 and restricted by p-value cutoff (left). However, the majority of significant sites had weak binding so general analysis was further restricted to the top 97% of the range of binding strength (right).

(B) RXR. An RXR peak set was restricted as in panel A.

(C) H4K5Ac. A peak set was compiled using the MACS2 narrow peaks algorithm and restricted as in A. These peaks comprise subregions of fields of H4K5Ac so that 2 adjacent peaks roughly correspond to single enhancers between them.

In all plots, CAR and RXR tag pileups were quantified and normalized to the strongest peak, defined as 1000. Tag density vs p is shown in a log plots, and tag density of control vs treated peaks linear plots.
Figure S4. Comparison of peak subsets and linked gene expression

(A) Expression ratios of CAR-linked genes. Subset genes showed comparable stimulation or inhibition. 3 hr and 24 hr avg upregulation were similar, but downregulation was greater in the 24 hr avg.

(B) Transcript number increments. Distributions of incremental change were generally similar, except that Single up genes and more distant genes in dual regulation showed weaker effects.

(C) Pileup and (D) stimulation of CAR binding. Medians of Single up, and all peaks linked to down-regulation, were 10 – 15% less than those linked to upregulation.

(E) Distributions of CAR peak-promoter distances. The medians suggest preferred positions for peak-promoter distances: Gene 1, 17 kb and Gene 2, 64 kb for upregulation; 27 kb and 97 kb for downregulation. Peaks associated with single genes correspond to the positions for Gene 2 in the dual sets.

Tukey box plots; Genes 1 and 2, the closer and more distant in each pair, respectively; Subsets: Single, 1 gene/peak; Dual, 2 genes/peak; Opp, paired genes with opposite regulation
Figure S5. Analysis of DHS in peak-gene subsets

(A) Histogram analysis. Although hypersensitivity is apparent over CAR peaks, TCPOBOP treatment induced focal changes and a small decrease in DHS, particularly in association with downregulation. The changes suggest altered central binding rather than broader chromatin remodeling.

(B) Fractional change of subset DHS levels. The increments represent the fractional difference in DHS reads in a 100-bp window around the CAR peak summits. The DHS reads for individual CAR peaks before and after treatment were compared using paired T-tests. *, p<0.05, ***, p<0.001.
Figure S6. Regulation of gene expression by CAR-only sites

(A) Comparison of TSS distance for CAR-only and RXR:CAR peaks. The CAR-only peaks are moderately closer to promoters.

(B) Transcriptional contribution of unambiguous CAR-only regulation. The CAR-only analysis used peaks associated with genes that were not linked to RXR:CAR sites, 112 down and 58 upregulated genes. The plot shows relative transcription of the two closest genes to each CAR peak and demonstrates that CAR-only-linked transcription is a minor response, predominantly downregulation.

(C) Histogram analysis of CAR binding and H4K5Ac at CAR-only sites. The sites showed the expected binding of CAR. Upregulation was associated with increased H4K5Ac, which was not affected by downregulation, equivalent to correlations observed for RXR:CAR sites.

(D) Ontology profiles of genes regulated by CAR-only sites. The plots show significant correlations to two profiles, entirely through downregulation.
Figure S7. HNF4 analysis

(A) Peak set analysis. A MACS2 peak set was calculated from 4 HNF4α libraries. Treated and control Tag values were then quantified separately. Tag pileups under the calculated peaks were quantified and normalized to the strongest peak, defined as 1000.

(B) Peak set restriction. A linear plot compares pileups from the two experimental conditions. Cutoffs were calculated from the average of Control and TCPOBOP-treated libraries.

(C) Effects of HNF4α on induced H4K5Ac in peak subsets. The 6 CAR peak-gene subsets were divided into HNF4+ and HNF4- groups to compare levels of H4K5Ac before and after TCPOBOP treatment. HNF4 had a highly significant effect, but only in dual opposite peak sets. n, number of peaks in the subset group; r, ratios calculated from treated and untreated peak summits; p, p values calculated using paired T-tests of individual peak values. Boxed p values compare HNF4- and HNF4+ sets, using unpaired T-tests of the stimulation ratios of individual peaks.
TRANSPARENT METHODS

Animals
8-12 week female CD-1 mice (Charles River Laboratories, Wilmington, MA) were treated with TCPOBOP (Millipore Sigma, St. Louis, MO) 3 μg/g body weight in corn oil by gavage. The studies were confined to female mice for uniformity because there is moderate sexual dimorphism. Our prior research focused on females because they develop stronger proliferative responses to this treatment. All animal studies were conducted under protocols approved by the Institutional Animal Care and Use Committees of the University of Pittsburgh in accordance with National Institutes of Health guidelines.

ChIP-seq
Chromatin preparation and immunoprecipitation were carried out as previously described (Tian et al., 2011) with modifications. 3 hr after TCPOBOP treatment, mice were euthanized with inhaled isoflurane and livers rapidly dissected, minced, and treated with 1% formaldehyde in PBS for 10 min at room temperature. After neutralization with 0.125 M glycine for 5 min, liver cells were harvested and washed twice with cold PBS. The liver cell pellet was resuspended in 5 ml/0.5 g liver Cell Lysis Buffer (5 mM PIPES, pH 8.0, 85 mM KCl, 0.5% NP-40). Nuclei were liberated using a B Dounce homogenizer. The number of strokes was determined by microscopic visualization of small aliquots. For lysis, nuclear pellets were suspended in 2 ml/0.5 g liver Nuclear Lysis Buffer (50 mM Tris pH 8.1, 10 mM EDTA, 1% SDS) and incubated on ice for 20 min before sonication.

Nuclei were sonicated with a Diagenode Bioruptor at 4°C, using the high setting and 30 sec on/30 sec off cycling totaling 20-30 min to produce DNA fragments of 200-500 bp. Fragment size was verified by gel electrophoresis of DNA extracted from aliquots. All buffers were supplemented with Halt protease and phosphatase inhibitors (ThermoFisher).

Immunoprecipitation was carried out with the ChIP-IT high sensitivity kit (Active Motif) using chromatin derived from 15 mg liver and 5 μg antibody for all studies except CAR, which used 25 μg, for each IP. Decrosslinked ChIP DNA was processed with the Illumina TruSeq Nano DNA LT Sample Prep Kit for multiplex sequencing. Individual libraries ranged from 7 – 23 X 10⁶ reads. All ChIP-seq analyses combined multiple libraries for each experimental condition. Immunoprecipitations used the following antibodies. Anti-CAR1/2, sc-13065x; RRID:AB_2155209; anti-RXRα, sc-774x; RRID:AB_2270041, and anti-HNF-4 alpha, sc-8987x; RRID:AB_2116913 were obtained from Santa Cruz Biotechnology (Dallas, TX). anti-Histone H4K5ac, 39170; Rabbit anti-Histone H3K4me3, #39915; anti-Histone H3K9Ac, #39137; and anti-Histone H3K27Ac, #39133 were from Active Motif (Carlsbad, CA).

ChIP-seq study of CAR was limited by the availability of suitable antibodies. We previously characterized a series of CAR antibodies and found only one that worked for conventional ChIP (Tian et al., 2011). However, the antibody had blocking activity in gel shift assays, suggesting that the main epitope was within the DNA binding domain and thus inaccessible when CAR was crosslinked to chromatin. Presumably, the ChIP pulldown resulted from a minor epitope. ChIP-seq detection was optimized by titrating antibody concentration to the maximum level that increased peak detection over background. Data was then pooled from libraries of 7 independent livers to further increase sensitivity. Parallel analysis of control liver, which showed few CAR peaks, combined 4 libraries.
**RNA-seq and real time PCR**

Total liver RNA was prepared with the miRNeasy Mini Kit (Qiagen) and ribosomal RNAs were depleted with the Ribominus Eukaryote System (Life Technology) according to the manufacturer’s protocols.

Randomly primed first strand cDNA was synthesized with Superscript III first-strand Synthesis System, (Invitrogen) and real time PCR carried out with Absolute QPCR mix (Fisher). For RNA-seq, second strand cDNA was synthesized with the Superscript Double-Stranded cDNA Synthesis Kit (Fisher).

Double stranded cDNA was then sonicated with the Diagenode Bioruptor at 4°C, at low setting and 30 sec on 30 sec off cycling for 30 min, to produce DNA fragments of 150-800 bp. The cDNA was further processed with a TruSeq Nano DNA LT Kit for sequencing. The starting amount of total RNA for each RNA-seq library was 0.75 μg.

PCR primers:
- Cyp2b10, 5’-TCTGCTCCATTGTGTGGTA, 5’-GGCTGGAGAATGAGCTTATGA
- Cyp2b10us1, 5’-GGCTCCAGCCCTAGAGACCTACCAG, 5’-CGTGAAAGACACCAACCTGCACA
- Cyp2b10us2, 5’-GGCTCCAGCCCTAGAGACCTACCAG, 5’-CGTGAAAGACACCAACCTGCACA
- Cyp2b13, 5’-GATCCAACCTTTTTTCTCCAGTGTTT, 5’-ATTCAGCAACGCAGAACTGTTTCA
- Cyp2b9, 5’-CAGTGCTCCACGAGACTACATTGATACC, 5’-CTCCATAGTGAGGTTGCGCCTTG

**DNase-seq**

DNase-seq was modified from a published study (Grontved et al., 2012). Nuclei were isolated from fresh mouse liver as for ChIP experiments, homogenized with a size B Dounce in 30 volumes of DNase I digestion buffer (0.5 M sucrose, 10 mM Tris–HCl, pH 7.5, mM MgCl₂, 0.5 mM spermidine, 0.15 mM spermine), and digested with DNase I (Sigma) at 7 Kunitz units per 100 μl for 10 minutes at 30°C. 100-500 bp DNA fragments were purified from an Invitrogen 2% E-Gel 2% and further prepared as for ChIP-seq.

**Sequencing and data analysis**

Enriched DNA from ChIP-seq and DNase-seq libraries were analyzed with 50 bp single end, RNA-seq with 100 bp paired end, reads on an Illumina Hiseq 2500 at the Epigenetics Shared Facility of Albert Einstein College of Medicine in New York. Sequences were aligned to the mm9 reference genome through the bioinformatic pipeline at the same facility. For RNA-seq, transcript quantification, statistical testing, and annotation were performed using Cufflinks (Trapnell et al., 2012) or GenPlay (Lajugie and Bouhassira, 2011) programs.

**Filtering of non-analyzable genomic regions**

Initial ChIP-seq studies compared treated liver to control immunoprecipitations with normal serum. In the compiled sets, thousands of peaks were stronger than those associated with the most stimulated candidate genes. Visualization showed these strong peaks clustered to a few regions and were present in both CAR and normal-serum libraries. The latter, however, did not contain enough information to filter them. We therefore computationally enlarged the peak regions using GenPlay until they coalesced into regions and then visually adjusted their boundaries. The
nonanalyzable regions were compiled in a BED file as an mm9 artifact filter (Table S1). For ChIP-seq and DNase-seq, these regions were filtered from individual BAM files using BEDtools (Quinlan and Hall, 2010). The analysis also excluded chrY, chrM, and random unassembled genome segments.

Compilation and analysis of ChIP-seq peak sets
After filtering the BAM files, peak sets were generated using MACS2 (Feng et al., 2012). For CAR analysis, 7 treated libraries were combined to define peaks. For other NR, chromatin modifications, and DNase-seq, treated and untreated libraries were first combined together to define a peak set. Treated and untreated libraries were then combined separately with MACS2 and pileups quantified from each in GenPlay using the defined peak set.

Restriction of peak sets used only unbiased criteria: p-value and fraction of the total range of pileup values, usually excluding the bottom 3%. CAR analysis used an additional criterion, stimulation ratio, to exclude a few hundred peaks. MEME-ChIP analysis was used to validate the ratio cutoff, via detection of the RXR half site motif (Figure 2E), which was present in some peaks with a stimulation ratio < 1.7, but none with a ratio < 1.5.

Downstream analysis was carried out using BEDtools, the HOMER suite (Heinz et al., 2010), RnaChipIntegrator (https://github.com/fls-bioinformatics-core/RnaChipIntegrator), and the MEME suite (Bailey et al., 2015). In particular, generation of tag directories, histogram analysis, and Functional Enrichment Analysis used HOMER programs. De novo motif discovery used MEME-ChIP and subsequent scanning of these motifs used FIMO. For motif localization, random control sets were generated by the BEDtools program, Shuffle, matched by chromosome location, and length. Overlap of peaks was calculated using the BEDtools Intersect default setting (1-base overlap). For H4K5Ac, this method of overlap calculation was applied only to the data in Figure 2C. Subsequent analysis used HOMER calculations of H4K5Ac tag density within a 1000-bp window around CAR peaks.

Quantification of the hepatocyte transcriptome
The hepatocyte transcriptome was compiled from 9 RNA-seq libraries: 2 controls, 2 each after TCPOBOP treatment of 3 and 6 hr, and 1 each from treatments of 12, 18, and 24 hr. The libraries were quantified with GenPlay as raw exon reads that aligned with the Refseq annotation of 30,255 genes in the mm9 genome. Values and annotations for rRNA, mtRNA, and genes shorter than 130 bp were removed. The analysis also excluded chrY and random unassembled genome segments. Isoforms with duplicate gene names were also removed, retaining the isoform with the greatest number of reads. Of the resultant list of 24,000 unique annotations, 16,800 genes had measurable expression. Libraries then received simple linear normalization using the total number of reads. These values reflect transcript mass (as reads/transcript). Following normalization, the values were divided by molecular weight to reflect transcript number (as reads/kb/transcript).

For individual time points, expression was compared as ratios to control reads. A second parameter, average hourly expression over 24 hr (24 hr avg reads), was calculated from the whole set of libraries to provide a single parameter that represented the full time course ((3 X (r0 + r3)/2 + 3 X (r3 + r6)/2 + 6 X (r6 + r12)/2 + 6 X (r12 + r18)/2 + 6 X (r18 + r24)/2) / 24); r, reads; subscripts, hr after treatment.
For statistical analysis, the series of expression ratios for each transcript \((t = 0, 3, 6, 12, 18, \text{ and } 24)\) was compared to Ppiα mRNA using one-tailed paired T tests and was further refined by calculation of Benjamini-Hochberg FDR.

The data were also compared to 2 libraries from isolated control hepatocytes to filter out non-hepatocyte transcripts, defined as >80\% depletion compared to whole liver. The hepatocyte transcriptome of 12,440 genes encompassed a 5-log range of expression. mRNA number was calculated from the reads per gene of the most abundant transcript, \(Alb\), ~20,000 molecules/cell. Expression levels at the low end of the transcriptome represented approximately 0.1 transcripts per cell.

**Data and Software Availability**

Raw datasets were deposited in the NCBI SRA database: RNA-seq, SRP125909; ChIP-seq and DNase-seq, SRP125957. Raw datasets were deposited in the NCBI SRA database: RNA-seq, SRP125909; ChIP-seq and DNase-seq, SRP125957.

| acc    | name                                   | organism           | tag    | value |
|--------|----------------------------------------|--------------------|--------|-------|
| SRS2727669 | ChIPseq-CAR-CTL-rep-1               | Mus musculus       | strain | CD1   |
| SRS2727668 | ChIPseq-CAR-CTL-rep-2               | Mus musculus       | strain | CD1   |
| SRS2727666 | ChIPseq-CAR-CTL-rep-3               | Mus musculus       | strain | CD1   |
| SRS2727667 | ChIPseq-CAR-CTL-rep-4               | Mus musculus       | strain | CD1   |
| SRS2727665 | ChIPseq-CAR-TCP-rep-1               | Mus musculus       | strain | CD1   |
| SRS2727664 | ChIPseq-CAR-TCP-rep-2               | Mus musculus       | strain | CD1   |
| SRS2727662 | ChIPseq-CAR-TCP-rep-3               | Mus musculus       | strain | CD1   |
| SRS2727663 | ChIPseq-CAR-TCP-rep-4               | Mus musculus       | strain | CD1   |
| SRS2727670 | ChIPseq-CAR-TCP-rep-5               | Mus musculus       | strain | CD1   |
| SRS2727671 | ChIPseq-CAR-TCP-rep-6               | Mus musculus       | strain | CD1   |
| SRS2727688 | ChIPseq-CAR-TCP-rep-7               | Mus musculus       | strain | CD1   |
| SRS2727689 | ChIPseq-RXRa-CTL-rep-1              | Mus musculus       | strain | CD1   |
| SRS2727690 | ChIPseq-RXRa-CTL-rep-2              | Mus musculus       | strain | CD1   |
| SRS2727691 | ChIPseq-RXRa-TCP-rep-1              | Mus musculus       | strain | CD1   |
| SRS2727684 | ChIPseq-RXRa-TCP-rep-2              | Mus musculus       | strain | CD1   |
| SRS2727685 | ChIPseq-RXRa-TCP-rep-3              | Mus musculus       | strain | CD1   |
| SRS2727686 | ChIPseq-RXRa-TCP-rep-4              | Mus musculus       | strain | CD1   |
| SRS2727687 | ChIPseq-HNF4a-CTL-rep-1             | Mus musculus       | strain | CD1   |
| SRS2727681 | ChIPseq-HNF4a-CTL-rep-2             | Mus musculus       | strain | CD1   |
| SRS2727682 | ChIPseq-HNF4a-CTL-rep-1             | Mus musculus       | strain | CD1   |
| SRS2727677 | ChIPseq-HNF4a-TCP-rep-2             | Mus musculus       | strain | CD1   |
| SRS2727672 | ChIPseq-H3K4me3-CTL-rep-1           | Mus musculus       | strain | CD1   |
| SRS2727675 | ChIPseq-H3K4me3-TCP-rep-2           | Mus musculus       | strain | CD1   |
| SRS2727647 | ChIPseq-H3K4me3-TCP-rep-1           | Mus musculus       | strain | CD1   |
| SRS2727683 | ChIPseq-H3K4me3-TCP-rep-2           | Mus musculus       | strain | CD1   |
| SRS2727676 | ChIPseq-H4K5Ac-CTL-rep-1            | Mus musculus       | strain | CD1   |
| SRS2727679 | ChIPseq-H4K5Ac-CTL-rep-2            | Mus musculus       | strain | CD1   |
| SRS2727678 | ChIPseq-H4K5Ac-TCP-rep-1            | Mus musculus       | strain | CD1   |
| acc         | name               | organism     | tag | value |
|-------------|--------------------|--------------|-----|-------|
| SRS2725635  | RNA-seq-ctrl-rep-1 | Mus musculus | strain | CD1   |
| SRS2725634  | RNA-seq-ctrl-rep-2 | Mus musculus | strain | CD1   |
| SRS2725633  | RNA-seq-TCP-3h-rep-1 | Mus musculus | strain | CD1   |
| SRS2725632  | RNA-seq-TCP-3h-rep-2 | Mus musculus | strain | CD1   |
| SRS2725639  | RNA-seq-TCP-6h-rep-1 | Mus musculus | strain | CD1   |
| SRS2725638  | RNA-seq-TCP-6h-rep-2 | Mus musculus | strain | CD1   |
| SRS2725637  | RNA-seq-TCP-12h   | Mus musculus | strain | CD1   |
| SRS2725636  | RNA-seq-TCP-18h   | Mus musculus | strain | CD1   |
| SRS2725631  | RNA-seq-TCP-24h   | Mus musculus | strain | CD1   |
| SRS3489136  | CD1-Hepatocyte-rep-2 | Mus musculus | strain | CD1   |

**Published datasets analyzed in this study**

PPARα, ChIP-seq (GSE35262), expression (GSE8295); FXR, ChIP-seq (downloaded from the UCSC data base with the link from (Thomas et al., 2010)), expression (GSE57305); LXRα, ChIP-seq (GSE35262), expression (Boergesen et al., 2012); PXR, ChIP-seq (kindly provided by Dr. J. Cui, University of Washington), expression (GSE55746); THR, ChIP-seq (SRX879758), expression (GSE65947)
Supplementary References

Bailey, T.L., Johnson, J., Grant, C.E., and Noble, W.S. (2015). The MEME Suite. Nucleic Acids Res 43, W39-49.

Boergesen, M., Pedersen, T.A., Gross, B., van Heeringen, S.J., Hagenbeek, D., Bindesboll, C., Caron, S., Lalloyer, F., Steffensen, K.R., Nebb, H.I., et al. (2012). Genome-wide profiling of liver X receptor, retinoid X receptor, and peroxisome proliferator-activated receptor alpha in mouse liver reveals extensive sharing of binding sites. Mol Cell Biol 32, 852-867.

Feng, J., Liu, T., Qin, B., Zhang, Y., and Liu, X.S. (2012). Identifying ChIP-seq enrichment using MACS. Nat Protoc 7, 1728-1740.

Grontved, L., Bandle, R., John, S., Baek, S., Chung, H.J., Liu, Y., Aguilera, G., Oberholtzer, C., Hager, G.L., and Levens, D. (2012). Rapid genome-scale mapping of chromatin accessibility in tissue. Epigenetics Chromatin 5, 10.

Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell 38, 576-589.

Lajugie, J., and Bouhassira, E.E. (2011). GenPlay, a multipurpose genome analyzer and browser. Bioinformatics 27, 1889-1893.

Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841-842.

Tian, J., Huang, H., Hoffman, B., Liebermann, D.A., Ledda-Columbano, G.M., Columbano, A., and Locker, J. (2011). Gadd45beta is an inducible coactivator of transcription that facilitates rapid liver growth in mice. J Clin Invest 121, 4491-4502.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D.R., Pimentel, H., Salzberg, S.L., Rinn, J.L., and Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7, 562-578.