IMPACT OF TRANSCRIPTION FACTOR PROFILE AND CHROMATIN CONFORMATION ON HUMAN HEPATOCYTE CYP3A GENE EXPRESSION

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ABSTRACT:
Recent data have made it increasingly clear that the gene expression profile of a cell system, and its alteration in response to external stimuli, is highly dependent on both the higher order chromatin structure of the genome and the interaction of gene products in interpreting stimuli. To further explore this phenomenon, we have examined the role of both of these factors in controlling xenobiotic-mediated gene expression changes in primary and transformed human hepatocytes (HuH7). Using quantitative polymerase chain reaction, expression levels of several transcription factors implicated in the liver-specific regulation of the CYP3A gene family were examined in human adult and fetal liver RNA samples. These expression profiles were then compared with those obtained from both primary and transformed human hepatocytes, showing that, in general, cultured cells exhibit a distinct profile compared with either the fetal or adult samples. Transcriptome profiles before and after exposure to the CYP3A transcriptional activators rifampicin, dexamethasone, pregnane-16α-carbonitrile, and phenobarbital were subsequently examined. Whereas exposure to these compounds elicited a dose-dependent increase in CYP3A transcription in primary hepatocytes, no alteration in expression levels was observed for the hepatoma cell line HuH7. Alteration in the expression levels of pregnane X receptor and chicken ovalbumin upstream promoter transcription factor 1, and the disruption of higher order chromatin within HuH7 cells altered CYP3A expression and/or activation by xenobiotics toward that observed in primary hepatocytes. These data provide potential roles for these two processes in regulating CYP3A expression in vivo.

Both primary human hepatocytes and human hepatoma cell lines have been used for many years as research tools to understand the effects of stimuli on the liver, whether this be as a result of a pathobiological state or exposure to xenobiotics (Branden et al., 2003). However, it is widely recognized that neither of these systems is ideal, with both having potential limitations in relation to modeling the in vivo situation (Plant, 2004). For example, the expression of many proteins has been shown to decrease rapidly in cultured primary hepatocytes, with drug-metabolizing enzymes (DMEs) being particularly sensitive (Binda et al., 2003). Despite this, primary hepatocytes have been extensively used to study xenobiotic effects on DMEs, particularly at the level of the transcriptome (Raucy, 2003). By comparison, whereas hepatoma cell lines are capable of supporting xenobiotic-mediated transcription based upon nonchromosomal reporter genes (El-Sankary et al., 2001), evidence for genomic-based transcription is more equivocal (Krusekopf et al., 2003; Raucy, 2003; Usui et al., 2003).

An emerging concept is that of systems-biology, where cell-, organ-, or even organism-wide interaction networks are key in determining the body’s response to any single stimulus (Coulson et al., 2003). For example, xenobiotic-mediated transcription of drug-metabolizing enzymes such as the cytochromes P450 is known to be mediated via several ligand-activated transcription factors, including aryl hydrocarbon receptor, peroxisome proliferator-activated receptor, CAR, and PXR (Francis et al., 2003), and these transcription factors have been shown to interact with each other to determine the gene set expressed following xenobiotic exposure (Moore et al., 2000; Gonzalez and Carlberg, 2002). These interaction networks may have a major effect on determining cellular response to xenobiotics, both within (Barwick et al., 1996; Swales et al., 2003) and between (Barwick et al., 1996) species/cell types. In addition, alterations in higher order chromatin may also act as a powerful regulator of gene expression (Dillon and Festenstein, 2002), affecting both temporal (Thomassin et al., 2001) and tissue-specific expression (Boustedt et al., 2003) of genes, as well as the response(s) to chemical exposure (Song et al., 2004). To this effect, PXR, the primary activator of CYP3A gene expression, has recently been shown to bind to DNA in a chromatin-dependent manner (Song et al., 2004), providing a powerful example of how chromatin conformation may influence expression of genes such as the CYP3A subfamily. Changes in chromatin conformation are known to occur within cell lines (Esteller, 2003; Horikawa and Barrett, 2003), providing an alternative explanation for the differential gene expression/activation profiles seen between cell lines and in vivo tissue.

ABBREVIATIONS: DME, drug-metabolizing enzyme; ANOVA, analysis of variance; bp, base pair(s); CAR, constitutive androstane receptor; COUP-TFI, chicken ovalbumin upstream promoter transcription factor I; DMSO, dimethyl sulfoxide; GRα, glucocorticoid receptor α; HCA, hierarchical cluster analysis; HNF, hepatic nuclear factor; PCA, principal component analysis; PCN, pregnenalone-16α-carbonitrile; PXR, pregnane X receptor; Q-PCR, quantitative polymerase chain reaction; TSA, trichostatin A.
We have now further investigated the role of both transcription factor interaction networks and chromatin formation in determining the expression of CYP3A genes in human hepatocytes, and their transcriptional activation by xenobiotics. The human CYP3A subfamily consists of four functional members (CYP3A4, 5, 7, and 43); since these enzymes have both differing abundances and activities toward substrates (Gibson et al., 2002), it is important to understand how each family member is regulated and expressed in different hepatocyte sources. Using in vivo, primary and transformed hepatocytes, we have examined both the level of transcription factors implicated in CYP3A expression and the effect of chromatin status on basal and xenobiotoxic-mediated CYP3A expression.

Materials and Methods

Hepatocyte Sourcing and Culture. Human liver total RNAs, adult and fetal, were purchased from commercial suppliers, and primary human hepatocytes were obtained from the UK Human Tissue Bank, Leicester, UK. Clinical details of the donors are presented in Table 1.

Primary hepatocytes were cultured in William’s Medium E (containing 2 mM l-glutamine; JRH Biosciences, Lenexa, KS), 10% heat-inactivated newborn calf serum, penicillin/streptomycin/neomycin [50 U/ml, 50 μg/ml, and 100 μg/ml, respectively] and insulin (1 mg/ml) in collagen-coated 24-well plates (BD Biosciences, San Jose, CA) at 3 × 10^5 cells/well, allowed to attach for 24 h, and then exposed, in triplicate, for 48 h to varying concentrations of PCN, dexamethasone, rifampicin, phenobarbital, or vehicle (0.1% DMSO) for PCN, rifampicin, and phenobarbital; and 0.4% DMSO for dexamethasone, resulting in a total culture time of 72 h.

HuH7 cells, a human hepatoma cell line (Nakabayashi et al., 1982), were cultured in Dulbecco’s modified Eagle’s medium [containing 10% fetal bovine serum, 2 mM l-glutamine, 1X nonessential amino acids, and penicillin/streptomycin (100 U/ml penicillin, 100 μg/ml streptomycin)] in 25-cm² culture flasks at 37°C overnight. DNA was then purified and digested to produce promoter fragments of the indicated sizes (Table 2). Southern blots of the digested genomic DNA were analyzed with probes complementary to the regions ~951→~344 bp (CYP3A4), ~1036→~126 bp (PXR), and ~1108→~7 bp (GAPDH) of the respective target genes. The resultant autoradiograms were quantified using computer-based video densitometry (GeneTools; SYNGENE, Cambridge, UK).

The histone deacetylation inhibitor trichostatin A (TSA) was used to alter chromatin formation. HuH7 cells were pretreated for 1 h with either 0.1% DMSO or 250 nM TSA, and then incubated for a further 24 h with 0.1% DMSO or 250 nM TSA with or without 10 μM rifampicin. Total RNA was then extracted and Q-PCR analysis was then carried out as described previously.

Results

Basal Expression of CYP3A Enzymes in Human Hepatocytes. Q-PCR was used to examine relative expression levels of CYP3A transcripts in various sources of human hepatocytes (Fig. 1A). Hierarchical cluster analysis (HCA) was then performed on the dataset to determine the overall relationship of the various CYP3A expression profiles to each other. Figure 1B shows that primary human hepatocytes, cultured for 24 h, closely resemble adult in vivo expression, with respect to CYP3A expression (Pearson coefficient >0.9). However, cells cultured for 72 h no longer resemble the adult in vivo levels (Pearson coefficient <−0.5) but more closely resembles the HuH7 hepatoma cell line (Pearson coefficient >0.6).

Table 2: Genomic DNA fragments used for DNase I sensitivity assay

<table>
<thead>
<tr>
<th>Digest</th>
<th>Target Gene</th>
<th>Fragment Location</th>
<th>Fragment Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI/Hpal</td>
<td>CYP3A4</td>
<td>−4729 to +890</td>
<td>5619</td>
</tr>
<tr>
<td>BamHI/Hpal</td>
<td>CYP3A5</td>
<td>−5782 to +1622</td>
<td>7404</td>
</tr>
<tr>
<td>BamHI/Hpal</td>
<td>CYP3A7</td>
<td>−2050 to +2415</td>
<td>4465</td>
</tr>
<tr>
<td>HindIII/ClaI</td>
<td>PXR</td>
<td>−5124 to +208</td>
<td>5332</td>
</tr>
<tr>
<td>StfI/BclII</td>
<td>GAPDH</td>
<td>−6431 to +156</td>
<td>6587</td>
</tr>
</tbody>
</table>

To decrease COUP-TFI levels, HuH7 cells were transfected with 3 pmol of SMARTpool siRNA for COUP-TFI (MWG Biotech) using the TransIT-TKO transfection reagent (MWG Biotech), according to the manufacturer’s protocol. Cells were incubated with the transfection complex for 24 h, followed by 24 h of incubation with complete medium, containing either rifampicin or vehicle alone (0.1% DMSO). Q-PCR analysis was then carried out as described previously.

Analysis of Chromatin Conformation. To determine local chromosome conformation around target genes, DNase I sensitivity was used. Isolated intact nuclei were extracted by the method of Ashe et al. (1997) and incubated with RQ1 DNase (Promega) (0, 0.05, 0.1, 0.4, and 1 U) for 10 min at 37°C. Stop buffer (0.6 M NaCl, 20 mM Tris, 10 mM EDTA, 1% SDS, 0.5 mg/ml proteinase K) was added and samples were incubated at 37°C overnight. DNA was then purified and digested to produce promoter fragments of the indicated sizes (Table 2). Southern blots of the digested genomic DNA were analyzed with probes complementary to the regions ~951→~344 bp (CYP3A4), ~1036→~126 bp (PXR), and ~1108→~7 bp (GAPDH) of the respective target genes. The resultant autoradiograms were quantified using computer-based video densitometry (GeneTools; SYNGENE, Cambridge, UK).

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implicated in regulating CYP3A gene expression (Fig. 2). Levels of the nuclear hormone receptors PXR, CAR, and GR were substantially different between hepatocyte sources, with respect to both their absolute amounts and their relative abundance. Although expression of all three factors was significantly lower in HuH7 cells compared with in vivo adult, the overall expression profile showed the highest correlation, with a Pearson coefficient $>0.85$ (Fig. 2A).

Many nuclear hormone receptors utilize RXR proteins as heterodimerization partners, and hence, the expression of RXR was examined (Fig. 2B). RXR is the most abundant form in all the hepatocyte sources tested, followed by RXR and, finally, RXR. Again, HuH7 cells provided the highest correlation to the expression profile seen in adult in vivo hepatocytes (Pearson coefficient $>0.95$).

Q-PCR analysis of the hepatocyte sources for general transcription factors implicated in CYP3A gene expression demonstrated substantial variation (Fig. 2C). In contrast to the high correlation between HuH7 cells and in vivo adult tissue observed for the ligand-activated transcription factors and their heterodimerization partners, no significant correlation was seen in the levels of general transcription factors (Pearson coefficient $< -0.3$).

To examine the overall profiles of transcription factors within the various hepatocyte sources, a HCA dendogram derived from the entire transcription factor dataset was created (Fig. 3). Fetal and adult in vivo hepatocytes share the closest overall profile, with primary hepatocytes (24 h and 72 h) forming a second cluster; HuH7 cells are poorly correlated with both of these clusters.

**Effect of Xenobiotics on CYP3A Expression in Human Hepatocytes.** Primary hepatocytes or HuH7 cells were exposed to PCN, dexamethasone, rifampicin, or phenobarbital at a range of concentrations, and CYP3A expression profile was determined. Timing of exposure period was designed such that the start and the end (after 48 h of exposure) corresponded to the 24-h and 72-h time points used in the basal measurements (Fig. 2).

For all compounds, activation of transcription of at least one CYP3A was observed in primary hepatocytes. However, the extent and magnitude of this activation varied. For PCN, statistically significant increases in mRNA were observed for both CYP3A4 and CYP3A7 (Fig. 4A), producing increases of 242% and 601% of control, respectively at 50 μM. The high concentration required to elicit any response is consistent with the observation that PCN is not considered an activator of human CYP3A gene transcription, being specific for rodent PXR. The observed activation occurs only at the top dose and may be reflective of either the poor EC50 of PCN for human PXR (4 μM) or activation via a PXR-independent pathway. It should be noted that an increase in CYP3A4 transcript level was also observed, although this did not reach statistical significance ($p = 0.035$ and $p = 0.039$ for 10 μM and 50 μM, respectively). Rifampicin also caused a significant increase in transcript levels of CYP3A4 and CYP3A7 (511% and 238% of control, respectively, at 50 μM), whereas CYP3A5 and CYP3A43 levels were unaffected (Fig. 4B). By comparison, dexamethasone increased transcript level for all CYP3A enzymes, with a maximal activation of 2200% being observed for CYP3A4 at 50 μM (Fig. 4C). Such large increases are consistent with the literature for CYP3A4 (Pascussi et al., 2000), CYP3A5 (Hukkanen et al., 1998), and CYP3A7.
(Schuetz et al., 1993), although the observed activation of CYP3A43 (671% of control at 1 μM) is novel. The induction of CYP3As by dexamethasone can be seen to occur in a biphasic manner: below 1 μM, induction is probably via GRα, whereas at dexamethasone concentrations greater than 10 μM, the effect is most probably via a combination of GRα and PXR activation (Huss and Kasper, 2000). Exposure of primary hepatocytes to phenobarbital resulted in large increases in CYP3A4 expression levels (maximal 1200% of control; Fig. 4D), as previously reported (Raucy, 2003; Usui et al., 2003), with more modest changes in CYP3A5 and CYP3A43 (maximal 347% and 286% of control, respectively).

In comparison to the statistically significant increases observed in primary hepatocytes, exposure of HuH7 cells did not elicit any sta-
dexamethasone exposure did alteration in CYP3A transcript levels suggest statistically significant increases above control levels. Only in the case of some factors, PCA was repeated with the exclusion of both RXR and CAR/Sp1 representing the major variables within the dataset (25% and 20% of the overall variability, respectively). Recent evidence suggests that the ubiquitous transcription factor Sp1 is more likely to impact upon CYP3A gene expression at the level of xenobiotic-mediated responses and not basal expression (Bombail et al., 2004). To further refine the analysis to those factors which may regulate basal expression of CYP3A enzymes, PCA was repeated with the exclusion of both RXR-y and Sp1. As can be seen from Fig. 5B, this results in a slight alteration of the clustering, although HuH7 cells still remain separate from other hepatocytes. RXRα/PXR and COUP-TFI/PXR represent the major variables underlying this separation (41% and 24% of the variability, respectively, with >95% of the variability being accounted for in the first two axes of analysis).

Mechanisms Underlying CYP3A Expression Levels in Human Hepatocytes. PCA suggested that PXR expression is an important factor potentially underlying the observed differences in genomic activation between the nonpermissive HuH7 cell line and permissive primary hepatocytes. Since PXR expression is significantly lower in HuH7 cells than in other hepatocyte sources (Fig. 2A), HuH7 cells were cotransfected with a PXR expression plasmid to increase PXR expression, as assessed at both the transcript (Fig. 6A) and protein (data not shown) levels. Resultant levels of PXR were approximately 2.5-fold and 2-fold above those measured in vivo for transcript and protein levels, respectively. In addition, over-expression of PXR altered the ratio of PXR/COUP-TFI transcript levels, also identified by PCA as a driver of HuH7/primary hepatocyte differences, to approxi-
FIG. 4. Effect of xenobiotic exposure on CYP3A expression profile in primary hepatocytes and HuH7 hepatoma cell line. HuH7 cells and primary hepatocytes were exposed to various concentrations of PCN (A), rifampicin (B), dexamethasone (C), or phenobarbital (D) for 48 h. Total RNA was prepared and CYP3A enzyme expression was measured using Q-PCR as described under Materials and Methods. n.d., not detected. Each data point represents $n=3$, and statistical analysis was performed using a one-way ANOVA, with Bonferroni post hoc analysis. *, $p<0.05$; **, $p<0.01$ relative to vehicle control.
FIG. 5. Principal component analysis of relative transcription factor expression levels in human hepatocytes. Principal component analysis was carried out using all data gained from the various hepatocyte sources as described under Materials and Methods. A, PCA was carried out excluding only RXR values from dataset; B, PCA was carried out excluding both RXR and Sp1 values from the dataset.

FIG. 6. Alteration of PXR and COUP-TFI expression levels does not increase CYP3A4 expression in HuH7 cells or its transcriptional activation by xenobiotics. PXR expression was increased in HuH7 cells by transfection of the pSG5-hPXR expression plasmid, and PXR, COUP-TFI, and CYP3A enzyme expression was measured under basal (A) and rifampicin-induced (B) conditions using Q-PCR as described under Materials and Methods. n.d., not detected. C, COUP-TFI expression was decreased by transfection of HuH7 cells with SMARTpool siRNA for COUP-TFI as described under Materials and Methods. PXR, COUP-TFI (C), and CYP3A enzyme (D) expression was measured under basal and rifampicin-induced conditions using Q-PCR as described under Materials and Methods. Each data point represents n = 3, and statistical analysis was performed using a one-way ANOVA, with Bonferroni post hoc analysis. *, p < 0.05; **, p < 0.01 relative to control level.
spond to alterations in level of functional protein, but the magnitude of the change may be different due to post-transcriptional and translational events. Second, all of the probe/primer sets used for Q-PCR analysis in this study were designed to amplify the major transcript produced from each gene. They do not, however, distinguish between alternatively spliced transcripts: it is therefore possible that although the level of transcripts from any single gene does not alter between hepatocyte sources, the nature of the transcript (and hence, protein product) does, with switching between alternate transcripts occurring. Although probably only of minor relevance, such a possibility does represent an alternate explanation for phenotypic differences between the hepatocyte sources tested herein, and which would not be detected in this assay.

As expected, CYP3A4 represented the major CYP3A transcript expressed in adult in vivo tissue, whereas in fetal tissue CYP3A7 was the major transcript (Oesterheld, 1998). Primary cultures of adult human hepatocytes closely model the in vivo adult situation following 24 h of culture, with HCA showing a high correlation. Primary human hepatocytes following 72 h of culture exhibited significantly lower levels of CYP3A expression than either fresh primary cells or in vivo tissue, consistent with previous reports (Binda et al., 2003). In fact, CYP3A expression in 72 h hepatocytes most closely resembled the hepatoma cell line HuH7 in terms of both their absolute expression levels and overall profile. As primary cells used in experiments will almost certainly be cultured for at least 72 h this suggests that, with respect to CYP3A metabolic studies, HuH7 cells may be as applicable as primary tissue cultures. One important observation from these studies is the change in relative expression of CYP3A enzymes: In both primary cells cultured for 72 h and HuH7 cells, CYP3A5 is the most abundant transcript, as opposed to CYP3A4 (Fig. 1A). Since the enzyme characteristics of CYP3A4 and CYP3A5 are not identical (Williams et al., 2002), this should be taken into account when extrapolating metabolic data from both cultured primary hepatocytes and HuH7 cells to humans.

CYP3A basal expression profiles provide information on the relative closeness of a system to the in vivo situation, but do not provide mechanistic explanations for any differences, nor do they provide information on how transcriptional responses to xenobiotic exposure are modeled within the cells. To examine this, we studied the expression of ligand-activated transcription factors (CAR, PXR, GR), their heterodimerization partners (RXR), and general transcription factors (COUP-TFI, HNF3, HNF4, CCAAT/enhancer binding protein α, Sp1), which have been implicated in the expression of CYP3A enzymes. Considerably more variation was observed between the hepatocyte sources with regard to expression of these transcription factors than was observed with the CYP3A enzymes. Interestingly, primary hepatocytes cultured for 24 h showed no correlation with adult levels of ligand-activated transcription factors (Pearson correlation <0.1), primarily due to significantly higher levels of PXR in the primary cells. Since CYP3A profiles between these two hepatocyte sources were highly correlated, this finding strongly suggests that the CYP3A profile cannot be wholly attributable to expression of ligand-
activated transcription factors. Such data are consistent with the known weak correlation between the levels of GRs transcripts and CYP3A4 transcripts/activity previously observed (Usui et al., 2003).

Heterodimerization of ligand-activated transcription factors predominantly occurs with RXRα, and this is highly abundant in all samples tested; limitation of heterodimerization partner is therefore unlikely. Heterodimers with RXRβ and RXRγ are also possible, although generally less active (Pfahl et al., 1994), potentially allowing control through sequestration of ligand-activated transcription factors in less active/inactive complexes. However, because expression of these factors in all hepatocyte sources is similar (RXRα > RXRβ > RXRγ), it appears unlikely that any such control point would have a major effect.

Surprisingly, HuH7 cells showed the highest correlation with in vivo tissue for expression of the ligand-activated transcription factors and their heterodimerization partners. Such data seem in conflict with the observation of ourselves and others that hepatoma cell lines generally respond poorly to xenobiotic-mediated transcriptional activation of CYP3A expression, both at the genomic (Domanski et al., 2001; Krusekopf et al., 2003; Usui et al., 2003; Song et al., 2004) and reporter gene (El-Sankary et al., 2000) level, if no additional manipulations of the cell line are made. Two possible scenarios may underlie this observation; first, the absolute expression of these factors (significantly lower in HuH7 cells) is limiting. Second, another level of control exists that is disrupted in HuH7 cells. Cotransfection of an expression plasmid for PXR is known to markedly increase CYP3A reporter gene activation in hepatoma cells (El-Sankary et al., 2000, 2001), with coexpression of GRs further enhancing this response (El-Sankary et al., 2000), possibly through activation of the genomic copy of PXR (Pascussi et al., 2000; Song et al., 2004). Such data are not inconsistent with the first scenario, where genomic-mediated expression of transcription factors is qualitatively similar to in vivo tissue, but quantitatively too low to allow efficient expression of plasmid-based reporter genes. Indeed, experiments carried out in this study have shown that over-expression of PXR in HuH7 cells does restore rifampicin-mediated increases in CYP3A4 and CYP3A7 gene expression. However, no increase in basal expression of CYP3A4, 5, or 7 transcripts is observed, suggesting that increased expression of ligand-activated transcription factors alone is insufficient for normal expression of CYP3A genes. This is consistent with data from PXR null mice, which still express basal levels of CYP3A11, but are unable to undergo xenobiotic-mediated activation of this expression (Xie et al., 2000). It is interesting to note that PXR over-expression does, however, cause an increase in CYP3A43 expression in HuH7 cells, to nearly 400% of control values, although rifampicin-mediated increases are not observed (Fig. 6, A and B). CYP3A43 may therefore be under different control mechanisms to the rest of the CYP3A subfamily, due to either differential promoter sequences and/or chromatin conformation, although neither of these scenarios has been experimentally addressed. The spatial separation of the CYP3A43 gene from the rest of the CYP3A gene cluster, over 44 kbp and in the reverse orientation, may also play an important factor in such differential expression (Domanski et al., 2001).

Because COUP-TFI shows increased expression in HuH7 cells and has been shown to act as both a negative and positive regulator of gene expression (Tsai and Tsai, 1997), this factor may effect CYP3A expression. Reduction of COUP-TFI expression in HuH7 cells using RNAi did not alter expression of PXR, CYP3A4, CYP3A7, or CYP3A43, suggesting that it does not negatively regulate these genes. However, suppression of COUP-TFI levels did elicit a significant increase in CYP3A5 expression, suggesting that COUP-TFI acts to repress expression of at least one member of the CYP3A subfamily. COUP-TFI levels are significantly higher in the developing fetus (Fig. 2C), providing a potential mechanism by which CYP3A5 expression is repressed in the fetus, resulting in transcript levels 700-fold lower than that seen for CYP3A7 (Hakkola et al., 2001). Comparisons to fetal levels must be made with a degree of caution for two reasons: first, the data presented herein are derived from a single 18-week male sample and second, the levels of many transcript levels change dramatically during development (Stevens et al., 2003) and hence, the levels present herein are representative of only a single time point. However, the high levels of COUP-TFI observed in this study are in agreement with previous work (Miyajima et al., 1988; Pippaon et al., 1999). Whereas such a hypothesis is intriguing, it should be noted that it does not explain the low fetal expression of CYP3A4, suggesting that multiple levels of control exist to differentiate the expression levels of CYP3A subfamily members.

A final mechanism by which gene expression may be regulated is at the level of chromatin conformation (Alvarez et al., 2003). Gene expression rates are directly related to the degree of higher order chromatin, with even highly active promoters being repressed if placed in the wrong chromatin environment [e.g., heterochromatin (Kioussis and Festenstein, 1997)]. Many transcription factors may modify higher chromatin order (Rigaud et al., 1991), either directly or through the action of recruited cofactors (Dillon and Festenstein, 2002). DNase I sensitivity assays in HuH7 cells demonstrate that the chromatin conformation around both the CYP3A4 and PXR promoters is relatively closed, providing a potential explanation for their reduced basal expression in HuH7 cells compared with in vivo tissue. It is interesting to note that the bands representing CYP3A7 and CYP3A5 show higher degrees of DNase I sensitivity than either CYP3A4 or PXR, correlating with their higher expression levels in HuH7 cells. Such differences in DNase I sensitivity also suggest that the chromatin status across the CYP3A cluster is not consistent, with regions of more “open” and “closed” chromatin existing, providing a control mechanism for the differential expression of the CYP3A genes. TSA treatment of the global level of histone acetylation, a signature associated with open regions of chromatin (Berger, 2002), and the differential action of TSA on the basal expression of CYP3A genes is consistent with such a hypothesis. TSA treatment did not recover the xenobioto-activated expression of CYP3A4, however, suggesting another level of control, probably at the level of chromatin conformation of the CYP3A4 enhancer (the xenobiotic response element module). PXR occupancy of the distal and proximal PXR response element has been shown to differ, suggesting different chromatin at these two spatially distinct genome locations (Song et al., 2004). Alternatively, such effects could be mediated by the ligand-activated transcription factor profile in HuH7 cells. TSA treatment led to a decrease in the expression of PXR, CAR, GRs, and COUP-TFI; since these factors may be involved in the transmission of xenobiotic signals, decreases in any of them may prevent rifampicin-mediated increases of CYP3A4 expression in cells with open chromatin at the CYP3A4 locus.

In summary, we have examined the transcriptome profile of various hepatocyte sources, and demonstrated fundamental differences between adult and fetal in vivo tissue, primary cells and the HuH7 hepatoma cell line. Furthermore, examination of the nonpermissive nature of HuH7 cells to genomic expression of CYP3A genes and their xenobiotic-mediated activation has suggested different levels of control for these two expression mechanisms. First, chromatin conformation plays an important role in CYP3A basal expression, with inhibition of histone deacetylation resulting in increased levels of CYP3A4 expression. Second, alteration of the levels of PXR and/or its ratio to COUP-TFI partially recovered the ability of HuH7 cells to support xenobioto-mediated genomic activation of CYP3A expres-
sion. It is thus attractive to hypothesize that a combination of these two factors are the main drivers underlying CYP3A expression in vivo.

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References

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