ACCELERATED PUBLICATION

Generation of hepatocytes expressing functional cytochromes P450 from a pancreatic progenitor cell line *in vitro*

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The proliferating AR42J-B13 pancreatic cell line is known to respond to glucocorticoid treatment by producing foci of cells that express the liver-specific albumin gene. We demonstrate that this cell line also expresses liver-specific or liver-enriched functional cytochrome P450 proteins when stimulated to trans-differentiate into hepatocytes by glucocorticoid. These data suggest that this cell line has an unusual ability to trans-differentiate into functional hepatocytes and that it could be possible to generate a limitless supply of functional hepatocyte-like cells *in vitro*.

Key words: cytochrome P450, liver, pancreas, stem cell, steroid.

INTRODUCTION

The parenchymal liver cell or hepatocyte is the most populous cell type of the liver. Hepatocytes are highly specialized both structurally and biochemically. They function as both exocrine cells (e.g. synthesis and secretion of bile constituents) and endocrine cells (e.g. secretion of many blood proteins), and have a prominent role in the metabolism of the body's carbohydrates, lipids and hormones. Many of these functions are essential for life, and hepatocytes are the only cells within the body able to perform them. Thus, when hepatocytes are damaged and liver function is significantly impaired, the only effective treatment for a patient is liver transplantation [1].

A major hurdle to the construction of an effective bio-artificial liver is the failure of hepatocytes to divide and/or retain differentiated phenotypes *in vitro* [2,3]. The transplantation or use of tissue from other species has therefore been considered, although this is controversial because of the risk of retroviral transfer [4]. Although the liver has a capacity to regenerate after necrosis through hepatocyte proliferation *in vivo*, this response has yet to be fully understood and does not occur to any great extent *in vitro* [5]. Indeed, a common response of primary hepatocytes to culture systems is a rapid de-differentiation to a phenotype that fails to significantly express liver functions [5–7]. Hepatocytes do not tolerate subcultivation, and hepatocyte-like cell lines do not effectively mirror primary hepatocyte functions in terms of either range or level of function [5–7].

Cytochrome P450s (CYPs) are haem-thiolate proteins which have a prominent role in the metabolism of both endogenous and exogenous compounds [8] and are widely acknowledged to be a primary determinant in the toxicity and/or carcinogenicity of exogenous compounds, such as drugs [9]. Tosh and colleagues identified a rat pancreatic cell line (AR42J-B-13 cells) that expressed the liver-specific albumin gene in response to glucocorticoid treatment [10]. More recently, they have suggested that B-13 cells express CYP2B1/2 and CYP3A1 isoforms when treated with glucocorticoid [11] (although these forms are normally expressed at low levels in rat liver). However, CYP expression was only determined by immunostaining and was not compared with normal hepatocyte levels, nor were any functional analyses undertaken. We have therefore examined this cell line for its ability to express functional CYPs, since these enzymes perform the important function of drug metabolism in hepatocytes. Their rapid loss in primary cultures of hepatocytes is frequently used as a generic and stringent measure of hepatocyte differentiation [5–7]. We demonstrate that B-13 cells express liver levels of CYPs in response to glucocorticoid that is metabolically active, as determined by probe substrate metabolism and by imparting cell sensitivity to the hepatotoxin paracetamol. The effect of glucocorticoid on B-13 cells is therefore not specific to albumin expression (which is unique in its stability in cultured hepatocytes and is therefore not an optimal measure of liver differentiation [6]). To our knowledge, this is the first observation of high-level, long-term expression of functional CYPs in any cell line. These results also suggest that, in principle, it may be possible to generate functional hepatocytes *in vitro*, a possibility that will have a major impact in the study of liver tissue development and the treatment of liver diseases.

MATERIALS AND METHODS

Animals, cell preparation and culture

Male Sprague–Dawley rats (250–300 g body mass) were bred in house at the Biological Services Unit, University of Aberdeen, Foresterhill, Aberdeen, Scotland, U.K. Rat hepatocytes were prepared by collagenase perfusion essentially as described in [12]. Hepatocytes were cultured in William's Medium E, supplemented with 1 μg/ml bovine insulin, 10 % (v/v) foetal calf serum (FCS),

Abbreviations used: ADD, androstenedione; B-13, AR42J-B13 cell line grown under normal conditions; B-13/H, AR42J-B13 cells after treatment with dexamethasone; CYP, cytochrome P450 – the cytochrome P450 nomenclature used is that outlined by Nelson et al. [8]; FCS, foetal calf serum; MET, metyrapone; β-NF, β-naphthoflavone; PB, phenobarbitone; PCN, pregnenolone 16α-carbonitrile; RT, reverse transcriptase; T-2α-OH (etc.), 2α-hydroxytestosterone (etc.).

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80 units/ml penicillin and 80 μg/ml streptomycin on collagen-type-I-coated plates. After 2 h, the medium was replaced with medium without FCS and insulin supplementation. B-13 cells were originally obtained from Dr D. Tosh (Developmental Biology Programme, University of Bath, Bath, U.K.) and were routinely cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS, 80 units/ml penicillin and 80 μg/ml streptomycin on plastic dishes. Further treatment was made by addition of compounds directly to the medium or by addition from 1000× concentrated stock solutions. Controls contained 0.1% (v/v) vehicle solvent.

Reverse transcriptase (RT)-PCR

Total RNA was isolated from cell preparations using Trizol (Gibco) according to the manufacturer's instructions. RT-PCR for the identification of CYP2C11 mRNA was performed essentially as previously outlined in [13] using the CYP2C11US1 (5′-GAAGCTGCCATGGATCCAGT-3′) and CYP2C11DS1 (5′-GGAGAAACGCCGGATCTCCTT-3′) primers. Amplified DNA was analysed on ethidium bromide-containing gels. Fragments were analysed further by sequencing to confirm that the correct sequence had been amplified using a BigDye® Terminator Cycle Sequencing Kit (PE Biosystems, Warrington, Cheshire, U.K.) and an Applied Biosystems sequencing machine.

Western blotting

Western blotting was performed after SDS/PAGE (9% gels) under reducing conditions using a MiniP2 Bio-Rad electrophoresis apparatus. Protein was transferred on to nitrocellulose and blocked overnight with 3% (w/v) milk protein and 0.3% (w/v) Tween 20. Anti-peptide antibodies raised against the C-termini of CYP3A1/3A23 (Ile-Ile-Thr-Gly-Ser) and CYP3A2 (Val-Ile-Asn-Gly-Ala) were used as described previously in [14]. The anti-CYP2C11 and anti-CYP1A1 antibodies were purchased from Daiichi Co. (Tokyo, Japan). The anti-CYP reductase and anti-amylase antibodies were obtained from Autogen Bioclear (Calne, Wilts., U.K.). The anti-CYP2E1 and anti-CYP2A antibodies were gifts from Professor M. Ingelman-Sundberg (Karolinska Institutet, Stockholm, Sweden) and Professor F. Guengerich (Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN, U.S.A.) respectively. After incubation with primary antibodies, blots were incubated with the appropriate horseradish-peroxidase-conjugated anti-IgG antibody. Detection was accomplished using the ECL® (enhanced chemiluminescence) kit (Amersham).

Testosterone metabolism and HPLC

Culture medium containing 250 μM testosterone was incubated with cells for 40 min before extraction of metabolites into dichloromethane. An internal standard (11β-hydroxytestosterone; T-11β-OH) was employed to control for inter-sample extraction variability. Organic extracts were dried under nitrogen, reconstituted in water/methanol (3:1, v/v) and filtered before analysis by HPLC essentially as outlined in [15]. Known concentrations of authentic standards [purchased from Steraloids (Newport, RI, U.S.A.)] were used to quantify individual metabolites using a detection wavelength of 254 nm.

Spectrophotometric detection of CYP and haem

Sodium dithionite reduced–CO versus sodium dithionite reduced difference spectra were performed in cell extracts essentially as outlined in [2]. Pyridine-haemochrome spectra were used to determine haem levels in cell extracts [16]. Protein concentrations were determined by the method of Lowry et al. [17].

RESULTS AND DISCUSSION

B13 cells undergo a proliferative and phenotypic alteration in response to glucocorticoid

B-13 cells readily proliferated under standard culture conditions when the medium was supplemented with serum (Figure 1A). Under these conditions, the cells formed colonies that were only loosely attached to the substratum (Figure 1C). However, when the medium was additionally supplemented with the glucocorticoid dexamethasone, many of the cells underwent a phenotypic alteration to a form referred to in the present paper as B-13/H cells. Over a period of 12 days, the cell population markedly reduced their rate of proliferation (Figures 1A and 1B), formed a more robust anchorage to the substratum, increased their size with respect to B-13 cells and transformed into a phenotype bearing a marked similarity to cultured hepatocytes (Figure 1D). The cells retained this phenotype for at least a month. The concentration of dexamethasone required to achieve these effects (EC_{50} between 1–10 nM; Figure 1B) is in agreement with the findings of Tosh and colleagues [10] and is commensurate with the affinity of dexamethasone for the rat glucocorticoid receptor [18].

Glucocorticoid-dependent phenotypic alteration in B-13 cells corresponds to expression of functional hepatic CYP proteins

CYPs are expressed in several tissues, although CYPs within families 1 to 4 are particularly highly expressed in the liver and are primarily associated with xenobiotic metabolism [9]. Figure 2(A) demonstrates that B-13 cells expressed undetectable levels of several constitutively expressed liver CYP isoforms, namely CYP2C11, CYP2A, CYP2E and CYP3A1. In contrast, treatment with 10 nM dexamethasone resulted in the expression of these isoforms within 5 days (note that oncostatin M was not added to medium to enhance trans-differentiation [9], because it is known to repress CYP expression [19]). After 12 days of dexamethasone treatment, the B-13/H cells were expressing significant levels of all four CYPs such that the levels of apoprotein expression were often similar to, or greater than, those seen in freshly isolated rat hepatocytes (Figure 2A). CYP3A2 expression was not observed in B-13 cells or in B-13/H cells within the first 12 days. However, CYP3A2 expression was observed in B-13/H cells after 30 days of treatment (Figure 2A). Amylase is an enzyme secreted by the pancreas and a marker for exocrine pancreatic cells [11]. The levels of cell-associated amylase were low in B-13 cells after trypsin passaging and increased in B-13 cells with time in culture (Figure 2B). However, plates containing B-13/H cells contained cells which expressed amylase at similar levels to pure B-13 cultures, suggesting that either B-13/H cells are able to express both pancreatic and hepatic genes or that only a subset of the B-13 cells responded to glucocorticoid and trans-differentiated into hepatocytes (which did not express amylase).

Fully functional cellular CYP activity requires a supply of NADPH, the synthesis of functional CYP reductase, and the synthesis and incorporation of the protoporphyrin IX prosthetic group into CYP proteins. Therefore, to determine if B-13/H cells were able to generate all the factors necessary for functional CYP activity, the metabolism of the CYP probe substrate testosterone [20] was examined in intact cultured hepatocytes, B-13 cells and B-13/H cells. Figure 3(A) indicates that rat hepatocytes hydroxylated testosterone at several positions, notably producing

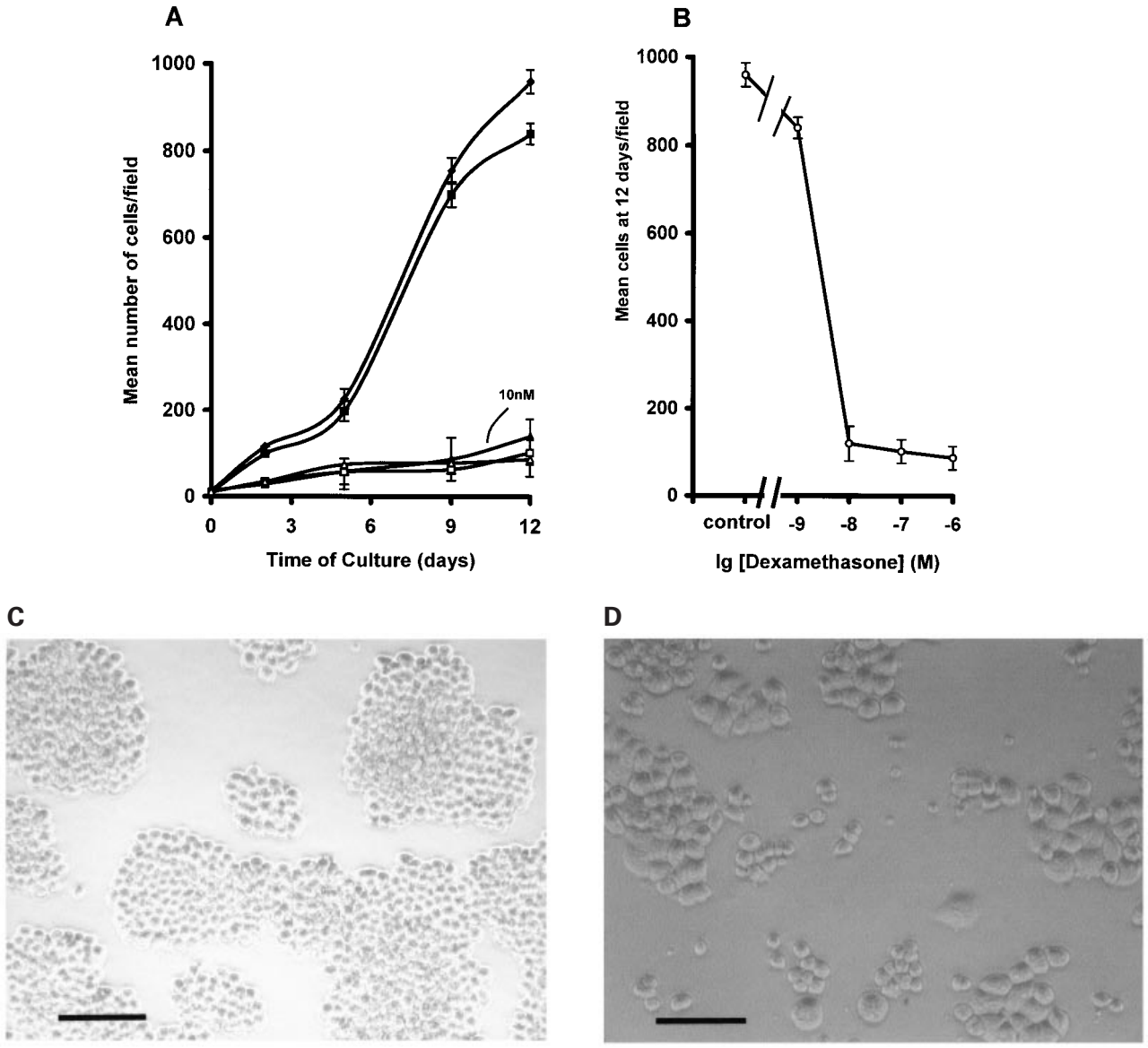

Figure 1 Effect of dexamethasone on the proliferation and morphology of B-13 cells: conversion into B-13/H cells

(**A**) and (**B**) B-13 cells were subcultured into 10 cm diameter culture dishes in standard Dulbecco's modified Eagle's medium culture medium (closed diamond) or with further supplementation with 1 nM dexamethasone (closed square), 10 nM dexamethasone (closed triangle), 100 nM dexamethasone (open square) or 1 μM dexamethasone (open triangle). The results show means $\pm$ S.D. of the number of cells visible under low-power magnification, counted in six randomly selected fields at various times of culture. Results are typical of seven separate experiments. Photomicrographs of control B-13 (**C**) and 1 μM dexamethasone-treated B-13/H cells (**D**) at 12 days of culture. The scale bar represents 100 μm.

16α-hydroxytestosterone (T-16α-OH) and 2α-hydroxytestosterone (T-2α-OH), metabolites produced by the major expressed male liver-specific CYP2C11 isoform [19]. The production of 6β-hydroxytestosterone (T-6β-OH) and 2β-hydroxytestosterone (T-2β-OH) are associated with CYP3A activity [20,21]. Low levels of 7α-hydroxytestosterone (T-7α-OH) were also detected and are likely to be associated with the expression of CYP2A1 [20,22]. The reduction of testosterone to androstenedione (ADD) was also observed in hepatocytes (Figure 3A). This reaction is mediated by several CYPs [20], but it is also mediated by other enzyme systems, such as 17β-hydroxysteroid reductase [23]. Table 1 demonstrates that the levels of testosterone hydroxylation rapidly fell in cultured hepatocytes such that only T-16α-OH was produced at detectable levels in hepatocytes after 3 days of culture. This observation agrees with numerous reports that rat hepatocytes undergo a reprogramming of gene expression during the isolation and early culture period that results in silencing of CYP gene expression [2–7].

B-13 cells showed little activity toward testosterone over the incubation period employed. Only production of relatively low levels of ADD and another unidentified metabolite were observed (Figure 3A). In contrast, B-13/H cells, after 30 days of treatment, produced high levels of several metabolites of testosterone (T-16α-OH; T-6β-OH and ADD) (Figure 3A). Interestingly, B-13/H cells produced the high levels of the CYP2C11-dependent T-16α-OH, but not the T-2α-OH, metabolite. It is known that there are several alleles of the CYP2C11 gene which show variations in testosterone metabolism (e.g. the Gunn rat strain

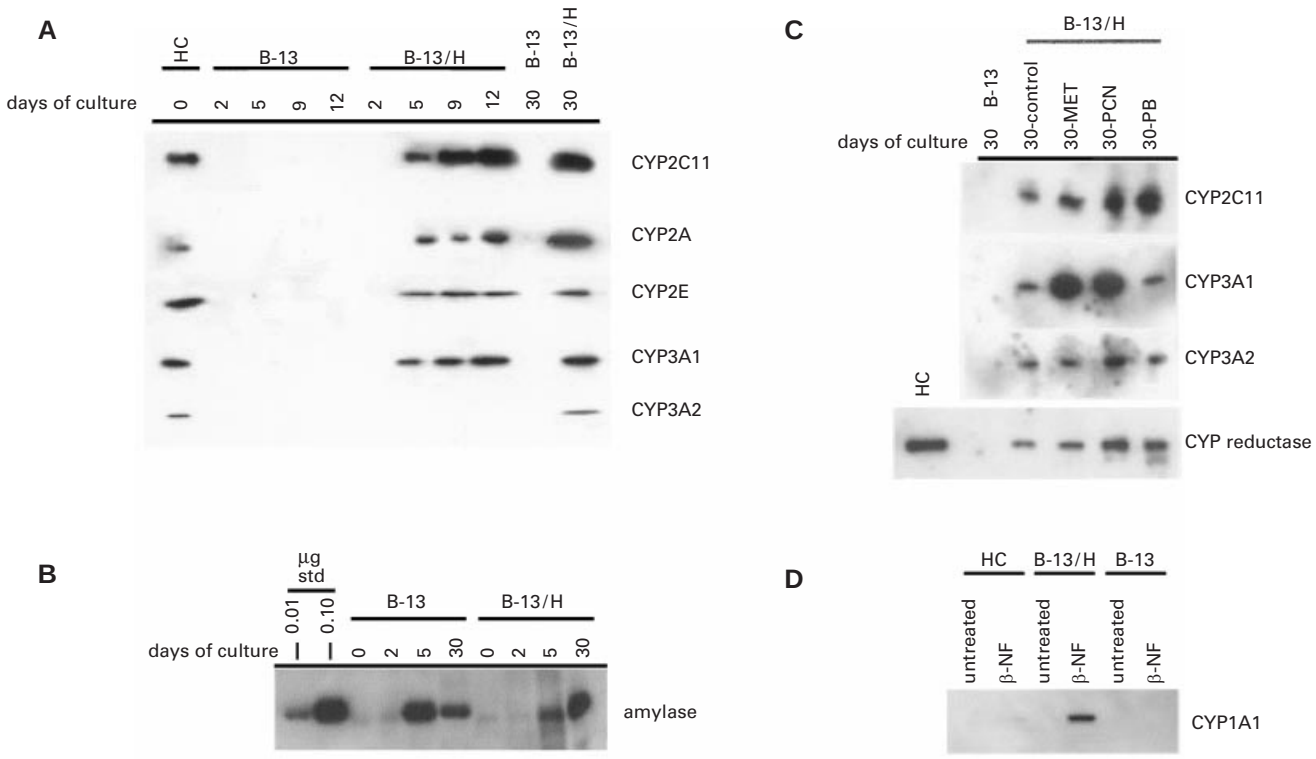

Figure 2 Expression of CYP proteins in freshly isolated hepatocytes, B-13 and B-13/H cells

(**A**) Expression of CYP in B-13, B-13/H and cultured rat hepatocyes. B-13 cells were cultured for the indicated number of days or with 10 nM dexamethasone to derive B-13/H cells. Whole-cell extracts were subjected to Western blotting as described in the Materials and methods section. HC, freshly isolated rat hepatocytes. All lanes contained 5 μg of protein. Results are typical of at least three separate experiments. (**B**) Expression of amylase in B-13 and B-13/H cells. The indicated extracts were probed with an antibody raised against α-amylase and which cross-reacts with rat and human proteins. std, purified human salivary α-amylase. All other lanes contained 10 μg of protein. Results are typical of three separate experiments. (**C**) Effect of MET, PCN and PB inducers on the levels of expression of CYP proteins in B-13/H cells. After 27 days of culture, B-13/H cells, stimulated to trans-differentiate with 10 nM dexamethasone, were treated with 500 μM MET, 20 μM PCN or 1 mM PB. Cells were treated with inducers daily for 3 days, with a daily medium change and then harvested on day 30. Control B-13/H cells were treated identically with inducer vehicle solvent. Whole-cell extracts were subjected to Western blotting as described in the Materials and methods section. B-13, cells cultured for 30 days without addition of dexamethasone. All lanes contained 5 μg of protein. Results are typical of at least three experiments. (**D**) Induction of CYP1A1 in B-13, B-13/H and cultured rat hepatocytes. Hepatocytes cultured for 2 h, or 30 day-cultured B-13 or B-13/H cells were treated with 20 μM β-NF or DMSO vehicle (untreated) for a further 24 h before analysis by Western blotting. All lanes contained 5 μg of protein. Results are typical of three separate experiments.

CYP2C11 has a dramatically reduced testosterone hydroxylation activity [24]). B-13 cells were originally isolated from the Lewis rat strain transplanted with an azaserine-induced pancreatic carcinoma [25]. CYP2C11 mRNA is detectable by RT-PCR in B-13/H cells (Figure 4), suggesting that the cells are expressing a CYP2C11 gene. It is possible, however, that the B-13 CYP2C11 gene is an allele whose product is only able to hydroxylate testosterone in the 16α position. When the production of testosterone metabolites is converted into total production and is normalized for cell protein, B-13/H cells demonstrated greater levels of testosterone metabolite production than hepatocytes after culture for 2 h (Table 1). Interestingly, B-13/H cells, but not B-13 or hepatocytes, produced a T-16β-OH metabolite, an activity that has been associated with a phenobarbitone (PB)-inducible CYP [26], although expression of CYP2B1/2 was not detected in our studies. These results therefore indicate that B-13/H cells not only express liver-specific or liver-enriched CYP apoproteins, but also support fully functional CYP activity, as indicated by the hydroxylation of testosterone.

Since B-13/H cells support high levels of functional CYP activity, extracts of B-13/H cells were examined for the classic reduced CYP–CO absorbance peak at 450 nm [27]. B-13 cells do not contain spectrophotometrically detectable CYP, whereas B-13/H cells contain a detectable peak, although the peak size is significantly lower than would be expected from the testosterone hydroxylation data (when compared with the levels of testosterone hydroxylation and levels of spectrophotometrically detectable CYP observed in hepatocytes; Figure 3B). The reasons for this disparity are not clear, but may be associated with CYP instability in B-13/H cells under the conditions of the spectrophotometric assay. Inactive CYP can form a reduced–CO absorbance at 420 nm [27], and low levels were evident in freshly isolated rat hepatocyte extracts (Figure 3B). However, haem and haem bound to other proteins will give rise to a reduced–CO spectral peak in the 410–420 nm region (e.g. albumin; results not shown). The peaks in absorbance at 410–412 nm in reduced–CO-treated B-13/H cell extracts therefore suggest that haem synthesis is occurring in the cells. Haem was readily detectable in B-13/H cells (40 ± 3.3 pmol/mg of protein compared with 350 ± 79 pmol/mg of protein in freshly isolated hepatocytes, see Figure 3C; haem was not detected in B-13 cells), but may be limiting the detection of spectrophotometrically detectable CYP, but not CYP activity. CYP functional activity is subject to rapid modulation (e.g. by phosphorylation [28]) and may be markedly

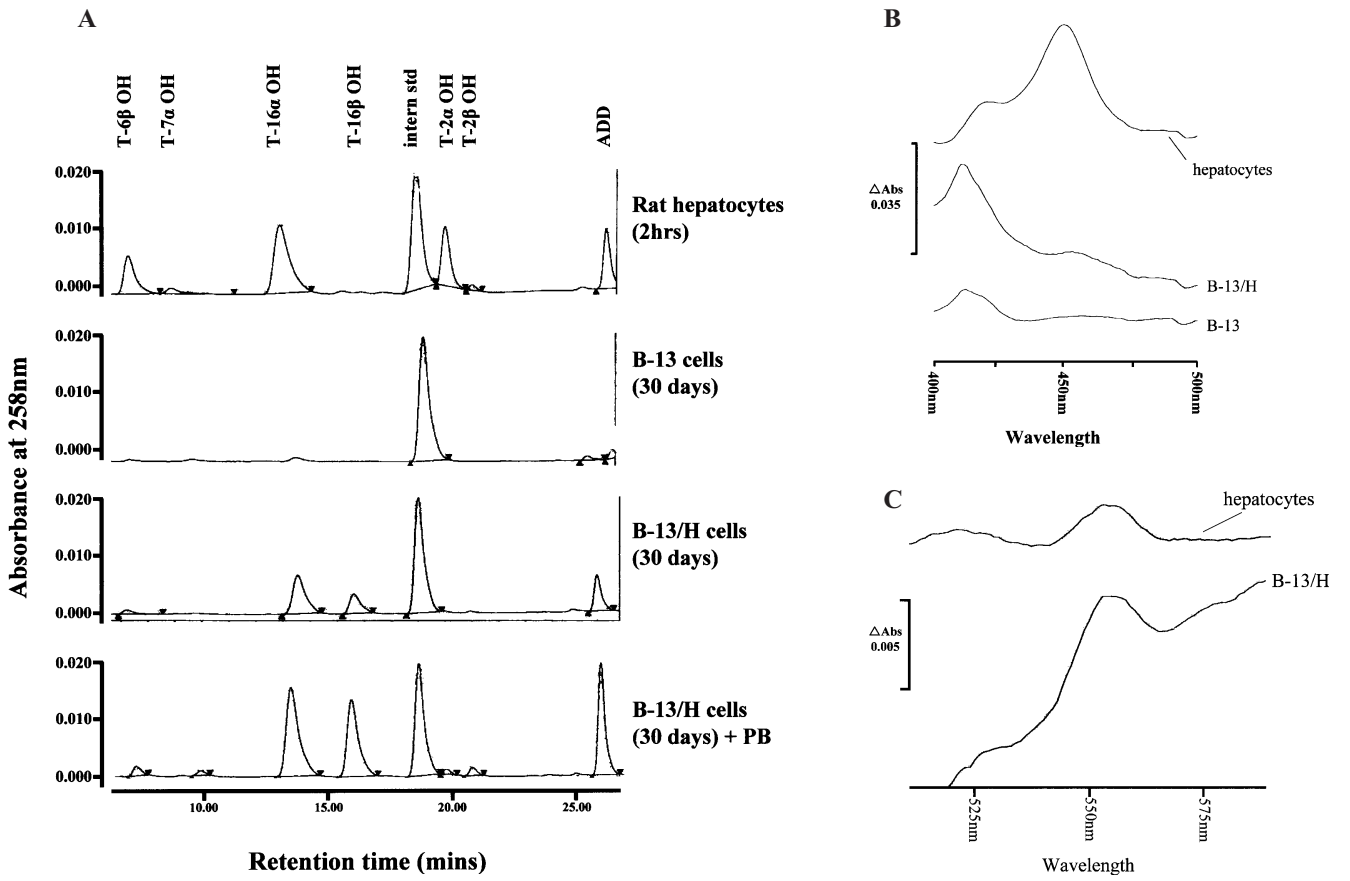

Figure 3 Functional CYP activity and spectrophotometric detection of haemoproteins and haem

(**A**) Testosterone metabolism in cultured rat hepatocytes, B-13 and B-13/H cells. Rat hepatocytes (3 mg of protein/flask) were cultured for 2 h as outlined in the Materials and methods section. B-13 (0.5 mg of protein/flask) and B-13/H (0.5 mg of protein/flask) cells were cultured for 30 days. Cells were washed with 1 × PBS and incubated at 37 °C with pre-warmed culture medium containing 250 μM testosterone for 40 min. Metabolite analysis was examined as outlined in the Materials and methods section. (**B**) Reduced–CO versus reduced difference spectra in rat hepatocyte, B-13 and B-13/H cells. Freshly isolated hepatocyte (2.1 mg/ml), 30 day B-13 cells (1.5 mg/ml) and 30 day B-13/H cells (2.6 mg/ml) solubilized extracts were scanned. Results are typical of two separate experiments. (**C**) Pyridine-haemochrome detection in hepatocytes and B-13/H cells. Hepatocyte (0.7 mg of protein/ml) or B-13/H cell extract (3 mg of protein/ml) (2 vol.) were mixed with 1 vol. of 0.5 M NaOH and 1 vol. of pyridine and the dithionite-reduced versus potassium-ferricyanide-oxidized spectra were determined [16]. B-13 cells did not contain a detectable pyridine-haem spectrum peak and is not shown. Results are typical of at least three separate experiments.

Table 1 Testosterone metabolism in rat hepatocytes, B-13 cells and B-13/H cells

Results are the means ± S.D. of the rate of metabolite production from three separate experiments. n/d, not detected.

	Production (pmol/min per mg of cell protein)								
	Hepatocytes		B-13 cells		B-13/H cells				
Metabolite	2 h	3 days	12 days	30 days	12 days	30 days	30 days + 500 μM MET	30 days + 20 μM PCN	30 days + 1 mM PB
T-16α-OH	38 ± 2.9	9.4 ± 1.05	n/d	n/d	83 ± 10.7	110 ± 14	310 ± 49*	130 ± 88	260 ± 51*
T-6β-OH	2.2 ± 0.15	n/d	n/d	n/d	n/d	18 ± 3.0	n/d	180 ± 10	035 ± 11.4
T-2α-OH	35 ± 2.5	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
T-7α-OH	1.0 ± 0.07	n/d	n/d	n/d	n/d	n/d	n/d	n/d	26 ± 7.6
T-16β-OH	n/d	n/d	n/d	n/d	23 ± 3.3	43 ± 10.2	46 ± 9.4	81 ± 52.4	160 ± 45*

*Significantly different rate of metabolite production ($P > 95\%$) compared with the rate of metabolite production by 30 day B-13/H cells using Student's t test (two tailed).

inhibited in hepatocytes in concert with the silencing of CYP gene expression that also occurs, although over a relatively longer time frame [6]. Alternatively, or in addition, the increase in CYP activity that can occur through the channelling of electrons from NADH to CYP via cytochrome b_5 [9] may be more active in B-13/H cells compared with hepatocytes. However, the cause of the high rate of testosterone hydroxylation by B-13/H cells remains to be determined.

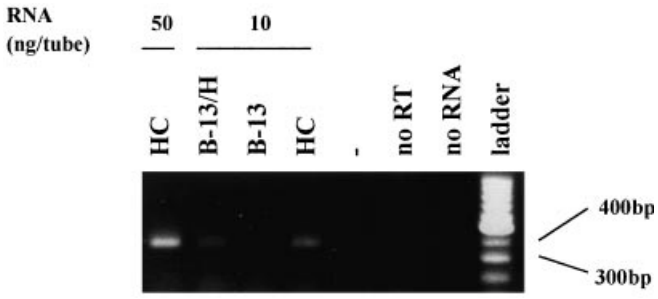

Figure 4 RT-PCR expression from CYP2C11 mRNA in freshly isolated hepatocytes, B-13 and B-13/H cells

RNA (ng/reaction as indicated) from freshly isolated rat hepatocytes (HC), 9 day B-13 or 9 day B-13/H cultures was subjected to RT-PCR analysis as outlined in the Materials and methods section and PCR products were subjected to gel electrophoresis. no RNA, reactions performed in the absence of RNA; no RT, reactions performed in the absence of RT; ladder, 100 bp ladder (Promega). Results are typical of three separate experiments.

Table 2 Effect of paracetamol treatment on the viability of cultured hepatocytes, B-13 and B-13/H cells

Hepatocytes cultured for 2 h and B-13 or B-13/H cells cultured for 30 days were then treated with 20 mM paracetamol for 24 h. The viability of attached and detached cells was assessed by 0.1 % (w/v) Trypan Blue exclusion. Similar results were obtained when attachment levels (viability) were used, as determined by a protein assay in culture wells after extensive washing (results not shown). Results are the means ± S.D. for three determinations from the same experiment, typical of three separate experiments.

	Cell death (%)	
Cells	Untreated	20 mM paracetamol
Hepatocytes	0.4 ± 0.1	87 ± 8.8*
B-13	2.8 ± 3.6	0.4 ± 0.7
B-13/H	1.8 ± 0.2	77 ± 7.4*

* Significantly different viability ($P > 95\%$) compared with untreated cells using Student's *t* test (two-tailed).

Induction of CYP protein expression

CYP expression is modulated in response to a number of xenobiotics such as metyrapone (MET), PB, pregnenolone 16α-carbonitrile (PCN) and β-naphthoflavone (β-NF) [12,29,30]. This response results in functional changes in the rate of metabolism of drugs and xenobiotics and is primarily regulated by receptor transcription factors (pregnane X receptor, constitutive activated receptor and aryl hydrocarbon receptor [29]). Table 1 (see also Figure 3A) indicates that the treatment of B-13/H cells with MET, PB and PCN resulted in increased hydroxylation of testosterone at several positions, whereas no effect on testosterone metabolism was seen in B-13 cells (results not shown). B13/H cells were particularly responsive to PCN and MET in that they stimulated an induction of CYP3A1 protein (Figure 2C), although there was a reduction in CYP3A-related T-6β-OH production in response to MET (Table 1) because, although MET is a transcriptional inducer of CYP3A1 in rats, it is also an inhibitor of CYP3A activity [30]. Interestingly, both MET and PB increase the levels of T-16α-OH production and the levels of CYP2C11 protein, whereas the effects of PCN are more variable (Table 1 and Figure 2C). The variable effects of PCN may be due to this compound acting as a glucocorticoid-receptor antagonist [18] and reversing the differentiating properties of dexamethasone. The 3.6-fold increase in T-16β-OH by PB treatment in B-13/H cells (Table 1 and Figure 2C) may be due to CYP2B expression, although we were unable to detect CYP2B expression in B-13 and B-13/H extracts by Western blotting (results not shown). CYP1A1 is expressed at constitutively low levels in hepatocytes, but expression is markedly up-regulated in response to aryl hydrocarbon agonists such as β-NF [12]. Figure 2(D) indicates that B-13/H cells were responsive to β-NF and up-regulated CYP1A1 expression, whereas B-13 cells did not respond. Interestingly, B-13/H cells were significantly more responsive to β-NF than were hepatocytes (Figure 2D), although this may be associated with the role of dexamethasone in B-13/H cultures potentiating CYP1A1 induction [12]. Taken together, these results indicate that B-13/H cells not only express CYP at levels seen in the liver, but are able to respond to several CYP inducers.

Sensitivity of B-13 cells to a hepatotoxic drug

To test functionality further, B-13 and B-13/H cells were challenged with an hepatotoxic drug that is dependent on metabolism by CYP for toxicity. Paracetamol is metabolized by CYP, primarily CYP2E, to an electrophilic metabolite NABQI that is responsible for the toxic effects of this drug [31]. Table 2 shows that paracetamol is toxic to hepatocytes and B-13/H cells, but not to B-13 cells. The expression of CYP2E1 in B-13/H cells is likely to account for the susceptibility of B-13/H cells to paracetamol and demonstrates that CYP expression in B-13/H cells is functional.

The liver and pancreas share a developmental origin in that they are both derived from the endoderm, with the pancreatic state being a default stage in hepatocyte phenotype development [32]. The present study and others [10,11] suggests that the B-13 cell line is a stem cell line clone that is able to differentiate into an hepatocyte phenotype in response to glucocorticoid exposure. Although previous work has identified that, under certain conditions (e.g. copper depletion [33]), hepatocytes become detectable within the pancreas, it is unlikely that such a trans-differentiation is normally regulated by glucocorticoids. However, the present study does suggest that it may be possible to generate an unlimited supply of hepatocytes from this cell line, and an understanding of how this process is possible in these cells will provide an insight into the regulation of liver tissue differentiation. The discovery or generation of a human equivalent would be of great value in the treatment of human liver disease, since it would have the potential to generate an unlimited number of hepatocytes *in vitro*, a process that has eluded scientists to date.

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