

Fibronectin-Mediated Hepatocyte Shape Change Reprograms Cytochrome P450 2C11 Gene Expression via an Integrin-Signaled Induction of Ribonuclease Activity

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ABSTRACT

A major limitation to the use of rat hepatocytes in the study of drug metabolism and toxicity is the rapid loss of CYPs. We demonstrate that the culture of rat hepatocytes results in a rapid loss of liver-specific CYP2C11 mRNA and transcripts encoding the general housekeeping gene copper-zinc superoxide dismutase (CuZnSOD) as well as poly(A⁺) mRNA. These losses are accelerated by fibronectin, which has no effect on the transcription of CYP2C11 and CuZnSOD. However, fibronectin, an extracellular matrix protein involved in cell adhesion and spreading, induces ribonuclease (RNase) activity. Fibronectin also increases hepatocyte diameter and data are presented that cell spreading is involved in the loss of both

CYP2C11 and CuZnSOD mRNAs. The use of functional blocking antibodies demonstrates that fibronectin is operating through its $\alpha_5\beta_1$ integrin receptor and genistein, a tyrosine kinase inhibitor, prevents hepatocyte spreading, RNase induction, and CYP2C11 mRNA loss. Collectively, the data indicate that hepatocytes in vitro actively promote the extinction of their phenotype via the autocrine effects of fibronectin rather than the current consensus that they simply lose differentiated function, such as CYP2C11 expression, through the absence of extracellular matrix proteins. The substrate specificity of the ribonuclease induced is also considered.

Hepatic CYPs (Nelson et al., 1996) are the major determinants of the pharmacological and toxicological activity of numerous drugs and many other foreign chemicals present in the human environment (Gonzalez, 1989; Paine, 1995). Therefore, a major limitation to the use of hepatocyte cultures in pharmacotoxicological studies is their rapid loss of CYP content (Paine, 1990). This loss of CYP and associated xenobiotic metabolism occurs in hepatocyte cultures prepared from the common species of experimental and farm animals as well as in human hepatocyte culture, suggesting a common underlying mechanism (Paine, 2000).

In adult male rat liver, a single CYP isoform, designated CYP2C11 (Nelson et al., 1996), constitutes the bulk of hepatic CYP content (Paine, 2000) and is responsible for the metabolism of endogenous steroids as well as a broad range of drug substrates (Morgan et al., 1985a,b; Morgan and Gustafsson, 1987). Here we demonstrate, in adult male rat hepatocyte cultures, that fibronectin, an extracellular matrix protein involved in cell adhesion and spreading, activates signaling pathways that rapidly control hepatocyte shape, RNase activity, and CYP2C11 mRNA content.

Materials and Methods

Cell Culture. Hepatocytes with a viability >85% were prepared from 250 to 290 g male Sprague-Dawley rats by collagenase perfusion (Wang et al., 1997). Cells (1.8×10^7) were routinely cultured on 150-mm diameter plastic Petri dishes in 20 ml of serum-free William's medium E (both from Flow Labs) as described previously (Wang et al., 1997) with treatments detailed in the figure legends. Bovine and rat plasma fibronectin was purchased from Sigma (St. Louis, MO), and the inhibitors used were of the highest purity available from commercial sources.

To inhibit hepatocyte spreading in culture, cells were cultured at a density of 5.4×10^7 viable cells/150-mm diameter Petri dish in 20 ml of culture medium, which is three times normal density. Complete inhibition of hepatocyte attachment to the culture plate substratum in the presence of 1 μ g/ml fibronectin was achieved by treating Petri dishes with 3 mg of polyhydroxyethylmethacrylic acid (PHEMA)/cm², as described previously (Folkman and Moscona, 1978).

Western Blotting for Detection of Medium and Cell-Associated Fibronectin. Hepatocytes were isolated and cultured without fibronectin and cells and culture medium collected at various times. In brief, medium was centrifuged at 1000g for 2 min at 4°C to remove cellular debris and 15 ml of supernatant was concentrated using Amicon centrifugal concentrators (30,000 mol. wt. cut-off). Hepatocyte monolayers were given five washes each of 20 ml of Williams medium E and then scraped into ice-cooled 20 mM Tris buffer, pH

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ABBREVIATIONS: PHEMA, polyhydroxyethylmethacrylic acid; SOD, superoxide dismutase; nt, nucleotide; CYP, cytochrome P450.

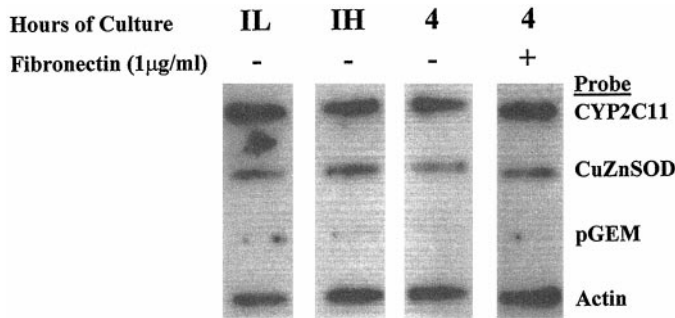


Fig. 1. Nuclear run-on transcription of CYP2C11, CuZnSOD, and actin genes in intact liver, isolated hepatocytes, and hepatocytes cultured for 4 h with (+) and without (–) fibronectin. Run-on transcription assays from intact liver (W), freshly isolated hepatocytes (IH), and hepatocytes cultured for 4 h \pm 1 μ g/ml bovine fibronectin. Radiolabeled RNA (10^7 cpm) from each preparation was hybridized to 0.25 pmol of the cDNA probes and pGEM plasmid as described previously (Wang et al., 1997). Results shown are typical of two separate experiments.

7.4, containing 250 mM sucrose and 1 mM dithiothreitol and homogenized using an Ultra-Turrax T-25 blender for 15 s. Aliquots were analyzed for protein using the Lowry assay and samples containing 20 μ g of homogenate protein or 120 μ l of concentrated culture medium were denatured with SDS under reducing conditions and subjected to SDS-polyacrylamide gel electrophoresis (6.8% separating gel/4% stack), blotted onto nitrocellulose membranes as described previously (Wang et al., 1997). Fibronectin was detected on blots by incubation with a purified rabbit anti-rat fibronectin polyclonal antibody (Chemicon International Inc., Temecula, CA) followed by horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad, Hercules, CA). Immunoreactive band chemiluminescence was detected on autoradiographic film using the enhanced chemiluminescence system (Amersham, Paisley, UK). Nuclear run-on transcription assays were performed as described previously (Wang et al., 1997).

RNA Isolation and Northern Blotting. Total RNA was isolated with RNazol B (Biogenesis, Bournemouth, Hants, UK) and subjected to Northern blotting as described previously (Wright et al., 1996). Blots were probed for poly(A⁺) mRNA levels using an oligo-dT₁₈

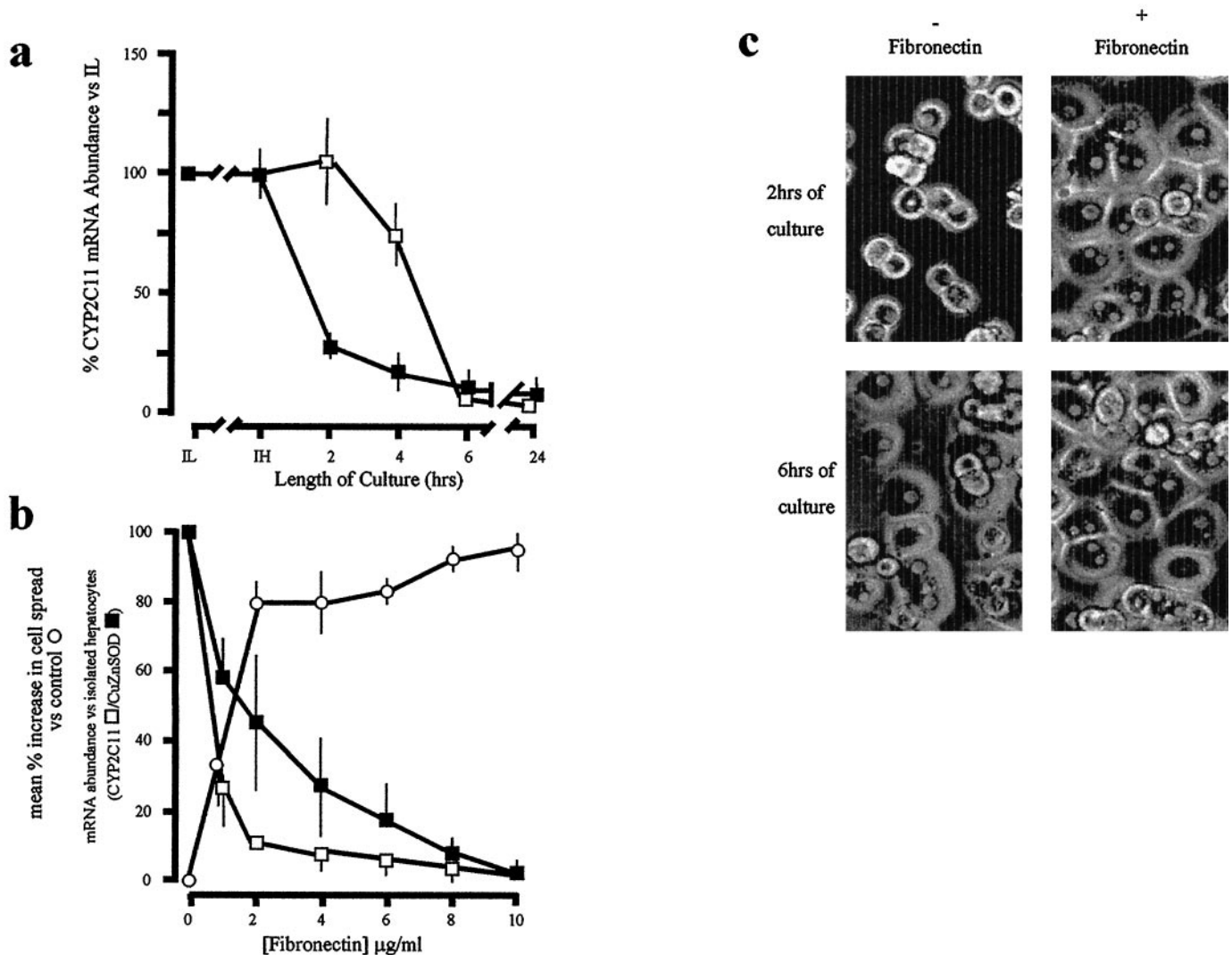


Fig. 2. Hepatocyte mRNA loss and spreading induced by fibronectin. a, time-course of CYP2C11 mRNA loss in hepatocytes cultured with (■) and without (□) 1 μ g of bovine plasma fibronectin (Sigma)/ml of culture medium. Percentage mRNA is presented as the abundance, normalized to 28S rRNA, compared with that present in the intact donor liver (IL). IH, freshly isolated hepatocytes. b, concentration-dependent effects of fibronectin on hepatocyte spreading (○) and levels of CYP2C11 (□) and CuZnSOD (■) mRNAs after 2 h of culture. Hepatocyte spreading is presented as the percentage of 200 hepatocytes in each treatment group that had spread to at least twice the diameter of hepatocytes cultured without fibronectin. c, photomicrographs of hepatocytes cultured for 2 and 6 h with (+) and without (–) 1 μ g of bovine fibronectin/ml.

probe (Pharmacia, Piscataway, NJ) labeled at the 5' end with [γ - 32 P]ATP and, for CYP2C11 and CuZn-superoxide dismutase (SOD) mRNAs, using randomly primed cDNAs (Wang et al., 1997) radiolabeled with [α - 32 P]dCTP using kits purchased from Promega (Southampton, UK). Blots were stained with methylene blue to determine the level of 28S rRNA. RNase activity was determined as described previously (Wang et al., 1997).

Data Analysis. Autoradiographs of Northern blots and photographic negatives of methylene blue stained gels for 28S rRNA determination were scanned by laser densitometry (Eagle Eye; Stratagene, La Jolla, CA) and analyzed using Phoretix software.

Determination of the concentration of fibronectin that produced a 50% effect on cell diameter as well as the effect of inhibitors on hepatocyte diameter and mRNA levels was determined by least-mean-squares nonlinear regression using the Enzfitter program (Elsevier Bioscience, Cambridge, UK). Student's *t* test was used to test statistical significance.

Results

When hepatocytes, isolated by dissociation of the liver with collagenase, are cultured they rapidly lose differentiated phenotype (Clayton et al., 1985; Rana et al., 1994; Runge et al., 1997) as typified by the loss of liver-specific CYP2C11 mRNA (Wang et al., 1997). However, the results presented in Fig. 1 demonstrate that hepatocyte isolation and culture for 4 h are without effect on the transcription of CYP2C11 and CuZnSOD, a general housekeeping gene. Nevertheless, both CYP2C11 and CuZnSOD mRNAs rapidly decline in hepatocyte cultures and their loss is accelerated by the addition of fibronectin to the culture medium (Fig. 2, a and b).

A recognized property of fibronectin is to make cells spread on plastic Petri dishes. Thus, after 2 h, hepatocytes cultured with fibronectin have a similar morphology as hepatocytes cultured for 6 h without fibronectin (Fig. 2c). The delay in spreading and CYP2C11 mRNA loss in untreated cultures is commensurate with the finding that hepatocyte isolation with collagenase depletes cell-associated levels of fibronectin and that these do not increase to levels comparable with the intact donor organ until after 4 to 6 h of culture (Fig. 3a). Similarly, examination of fibronectin levels in the culture medium indicates that hepatocytes take 4 h to secrete sufficient fibronectin to result in a medium concentration of 1 μ g/ml (Fig. 3b).

The results presented in Fig. 2b demonstrate that the ability of fibronectin, added exogenously, to promote hepatocyte spreading is dependent on its concentration, as is the loss of CYP2C11 and CuZnSOD mRNAs. Heat-denatured fibronectin (90°C for 60 min) did not promote hepatocyte spreading or mRNA loss. Both parameters share a median effective concentration for fibronectin of approximately 1 μ g/ml. That hepatocyte substratum attachment and spreading are involved in the loss of these mRNAs is demonstrated by preventing cell adhesion with PHEMA and by physically constraining spreading by culturing hepatocytes at three times the density that normally results in confluence. In both instances, fibronectin was unable to promote the loss of CYP2C11 and CuZnSOD mRNAs (Fig. 4, a and b). Finally, that fibronectin is operating through its $\alpha_5\beta_1$ integrin receptor (Ruoslahti, 1988) is demonstrated by the ability of antibodies that block the function of either the α_5 or β_1 subunits to prevent the loss of CYP2C11 and CuZnSOD mRNAs (Fig. 4c) as well as prevent spreading (data not shown), whereas antibody to the β_4 subunit, used as control, is ineffective.

To gain an insight into the signal transduction pathways that regulate cell shape changes and mRNA loss, we treated hepatocytes with a variety of cell-signaling inhibitors and found the results clustered into three groups (Fig. 5a). One group, typified by protein kinase A inhibitor and pertussis toxin as well as all of the solvent controls used, is composed of hepatocytes that have spread and lost their CYP2C11 mRNA. That hepatocyte spreading is involved in CYP2C11 loss is supported further by the population that has neither increased its diameter in response to fibronectin nor lost CYP2C11 mRNA. Exactly the same clusters were found for CuZnSOD mRNA abundance (Fig. 5b). No hepatocyte population that had not spread but lost CYP2C11 and CuZnSOD transcripts was observed. However, the existence of a population that has spread but not lost CYP2C11 or CuZnSOD mRNAs (Fig. 5a) implies that the ultimate mechanism involved in mRNA loss is distal to the spreading process. In this respect, the finding that inhibitors of transcription and translation prevent the loss of CYP2C11 and CuZnSOD transcripts (Fig. 5b) suggests that the hepatocytes need to synthesize an entity to lose these mRNAs. An obvious candidate is an RNase, especially because fibronectin promotes the loss of poly(A⁺) mRNA (Fig. 6a). The results presented in Fig. 6b demonstrate that fibronectin induces cellular RNase activity

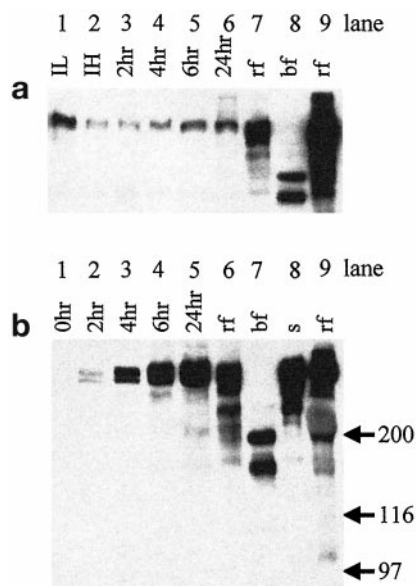


Fig. 3. Western blot of fibronectin levels in hepatocyte culture medium and hepatocytes in culture. a, levels of hepatocyte-associated fibronectin in intact liver and cultured hepatocytes. Lane 1, 20 μ g of intact liver protein (IL); lane 2, 20 μ g of isolated hepatocyte protein (IH); lane 3, 20 μ g of hepatocyte protein cultured for 2 h (2hr); lane 4, 20 μ g of hepatocyte protein cultured for 4 h (4hr); lane 5, 20 μ g of hepatocyte protein cultured for 6 h (6hr); lane 6, 20 μ g of hepatocyte protein cultured for 24 h (24hr); lane 7, 0.1 μ g of rat fibronectin (rf); lane 8, 0.1 μ g of bovine fibronectin (bf); lane 9, 1.0 μ g of rat fibronectin (rf). b, release of culture medium by hepatocytes into the culture medium. The concentrated equivalent of 120 μ l of medium was analyzed: lane 1, control medium (0hr); lane 2, medium after 2 h of culture (2hr); lane 3, medium after 4 h of culture (4hr); lane 4, medium after 6 h of culture; lane 5, medium after 24 h of culture (24hr); lane 6, 0.1 μ g of rat fibronectin (rf); lane 7, 0.1 μ g of bovine fibronectin (bf); lane 8, 30 μ g of rat serum protein; lane 9, 0.5 μ g of rat fibronectin (rf). Culture medium (supplemented with 100 μ g BSA/ml to block nonspecific binding) containing 0.25 μ g rat fibronectin/ml was added to culture plates without cells and incubated for up to 24 h to determine the degree of fibronectin binding to the culture plate, centrifugal elutriator, etc. No adherence was observed (data not shown). Arrows give position of protein molecular mass (kDa) markers.

and that this is blocked by genistein, which is also effective at preventing hepatocyte spreading and CYP2C11/CuZnSOD mRNA loss.

In contrast to the fibronectin mediated loss of CYP2C11, CuZnSOD, and poly(A⁺) mRNAs, the results presented in Fig. 7a show that the abundance of albumin mRNA remains relatively constant throughout a 24-h culture period. Similarly, incubation of extracts of hepatocytes treated with fibronectin mimic the culture situation by enhanced degradation of in vitro transcribed CYP2C11 mRNA but not of albumin mRNA (Fig. 7b).

Discussion

It has been known for many years that fibronectin, an extracellular matrix protein present in fetal calf serum, promotes hepatocyte attachment and spreading (Blaauboer and Paine, 1979). The current work demonstrates that the culture of rat hepatocytes with bovine fibronectin accelerates the loss of CYP2C11 and CuZnSOD mRNAs and that these changes are correlated with cell spreading. Over the 4-h time-frame studied, fibronectin accelerates the loss of CYP2C11 and CuZnSOD mRNAs without affecting the tran-

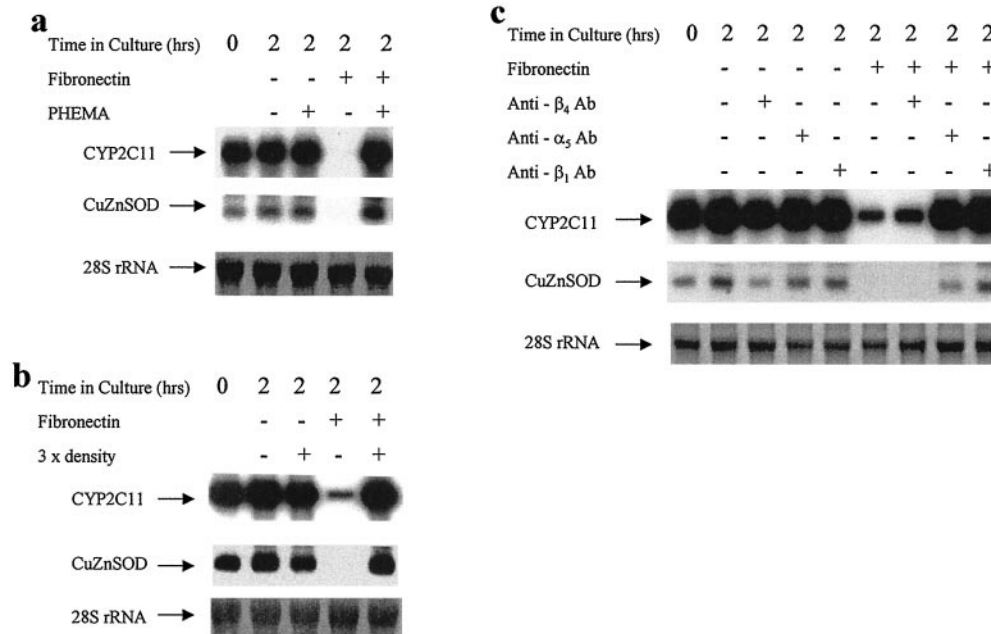


Fig. 4. Effect of preventing hepatocyte attachment (a), preventing hepatocyte spreading (b), and antibodies to integrin subunits (c) on CYP2C11 and CuZnSOD mRNA abundance in hepatocytes cultured for 2 h. a, Northern blot of RNA from hepatocytes cultured on plastic (-) or PHEMA-coated (+) Petri dishes with (+) and without (-) 1 μ g of bovine fibronectin/ml. b, Northern blot of RNA from hepatocytes cultured at 10^5 cells/cm² (-) or three times this density (+) on plastic Petri dishes with (+) and without (-) 1 μ g of bovine fibronectin/ml. c, Northern blot of RNA from hepatocytes cultured with (+) and without (-) 1 μ g of bovine fibronectin/ml and 5 μ g/ml of functional blocking antibody (Life Technologies, Gaithersburg, MD) to α_5 , β_1 , or β_4 integrins as indicated. All results typical of three separate experiments.

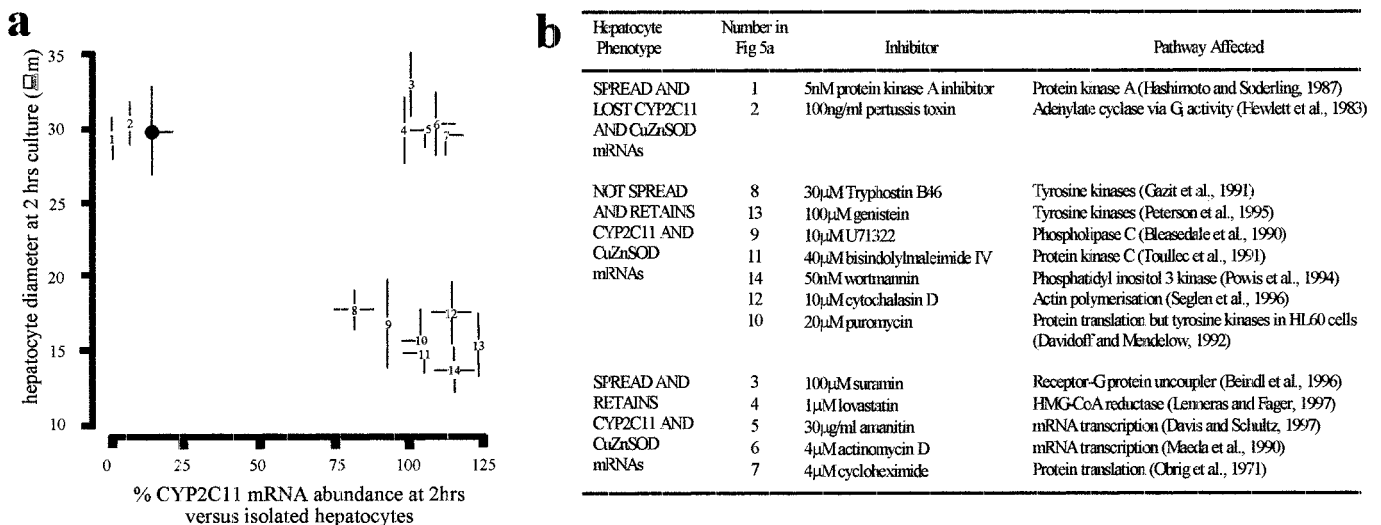


Fig. 5. Effect of signal transduction inhibitors on fibronectin mediated hepatocyte spreading and CYP2C11 mRNA abundance. a, scatter-plot comparing effects of cell-signaling inhibitors on hepatocyte diameter and CYP2C11 mRNA abundance in hepatocytes cultured for 2 h with 1 μ g of bovine fibronectin/ml. The diameter of 200 cultured hepatocytes in each treatment group was determined by phase contrast microscopy using a calibrated eye-piece graticule. Filled symbols, solvent controls. Results are the mean \pm S.D. of between 3 and 12 separate experiments. b, inhibitor concentrations used and resultant hepatocyte phenotype.

scription of these genes. Indeed, CuZnSOD mRNA abundance was chosen for study because its rate of transcription in hepatocytes is so slow that such rapid changes in its mRNA levels entirely reflect degradative events (Dougall and Nick, 1991). These changes in CYP2C11 and CuZnSOD mRNA abundance are mediated by fibronectin added either exogenously (Fig. 2) or synthesized (Odenthal et al., 1992) by the hepatocytes themselves (Fig. 3) which then seemingly acts in an autocrine fashion. Although other investigators (e.g., Scheutz et al., 1988; Niwa et al., 1996) have shown that qualitative changes in hepatocyte shape can prevent the loss of CYPs, the current work, to the best of our knowledge, is the first report to demonstrate that extracellular matrix proteins such as fibronectin actively promote the extinction of hepatocyte phenotype in vitro. In this respect we have found that fibronectin, through binding to a $\alpha_5\beta_1$ integrin activates a signal transduction pathway that leads to the loss of

CYP2C11 and CuZnSOD mRNAs. Thus, despite the different molecular weights of bovine and rat fibronectins (Fig. 3) because of splice variants and degree of glycosylation, both forms interact with the $\alpha_5\beta_1$ integrin receptor through an "RGD" domain (Ruoslahti, 1988). Accordingly, both rat and bovine fibronectins can activate the same signal transduction pathway, which, based on inhibitor studies, involves tyrosine kinases and, in view of the sensitivity to cytochalasin D (Fig. 5b), actin polymerization. However, the finding that hepatocytes can spread and not lose CYP2C11 mRNA (Fig. 5a) suggests that cytoskeletal rearrangements may only initiate the process. Because this "spread but not degraded" population is composed of hepatocytes treated with suramin, a receptor-G protein uncoupler (Beindl et al., 1996) and lovastatin, an inhibitor of ras function (Cuthbert and Lipesky, 1997), the pathway leading to CYP2C11 mRNA degradation possibly involves the participation of small GTPases subse-

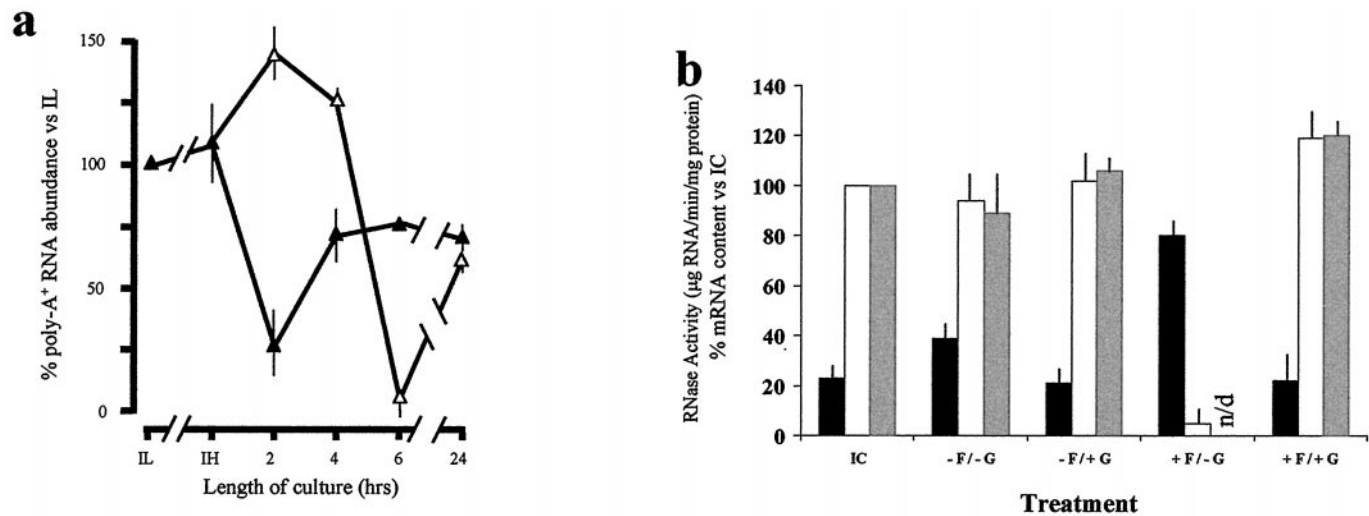


Fig. 6. Ribonuclease activity, CYP2C11, CuZnSOD, and poly(A⁺) mRNA abundance in hepatocytes cultured with fibronectin. **a**, time-course of poly(A⁺) mRNA loss in hepatocytes cultured with (▲) and without (△) 1 μg of bovine plasma fibronectin/ml of culture medium. Percentage mRNA is presented as abundance, normalized to 28S rRNA, compared with that present in intact donor liver (IL). IH, freshly isolated hepatocytes before culture. **b**, ribonuclease (RNase) activity (black bars) assayed in extracts of hepatocytes cultured for 2 h with (+) or without (-) 1 μg of fibronectin/ml (F) and 100 μM genistein (G) and compared with percentage of isolated cells (IC) CYP2C11 (open bars) and CuZnSOD (gray bars) mRNA levels. Results are the mean ± S.D. of three separate hepatocyte preparations. n/d, not detectable.

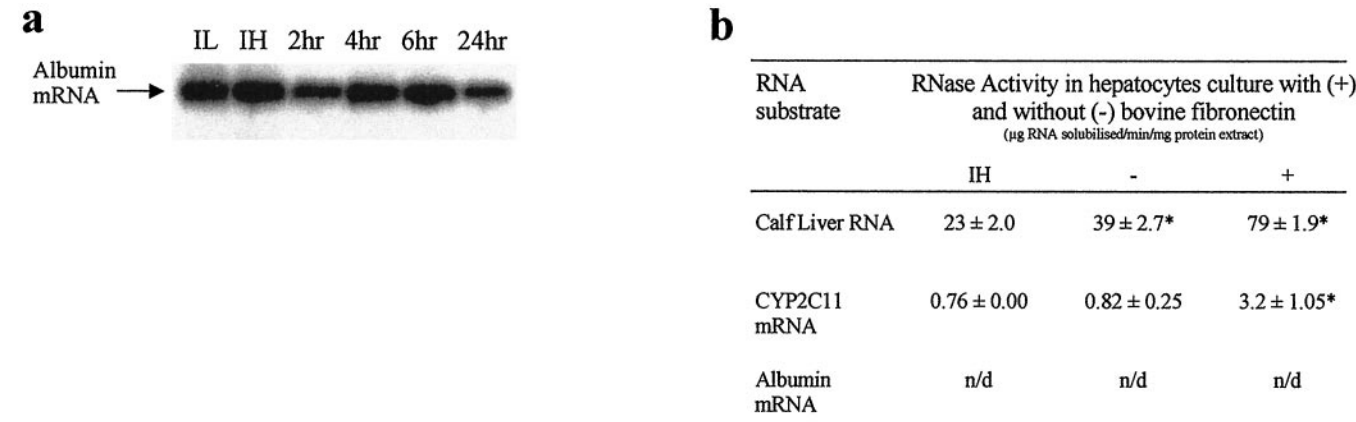


Fig. 7. Albumin mRNA abundance in hepatocytes cultured with fibronectin and the degradation of in vitro transcribed CYP2C 11 and albumin mRNAs. **a**, Northern blot of RNA from hepatocytes cultured with 1 μg of fibronectin/ml probed for albumin mRNA. **b**, RNase activity of hepatocyte extracts assayed (Wang et al., 1997) toward calf liver RNA, a 1956-nucleotide (nt) albumin mRNA and a 1865-nt CYP2C11 mRNA. Albumin mRNA can be predicted from the plasmid map to contain the full coding sequence, the 3'-untranslated region but no poly(A⁺) tail. CYP2C11 mRNA can be predicted to contain 5 nt of the 5'-untranslated region, the full coding sequence, the 3'-untranslated region and a 27-mer poly(A⁺) tail. Results are mean ± S.D. of three separate hepatocyte preparations. * denotes significantly different ($P > .05$) by Student's *t* test from freshly isolated hepatocytes (IH) and hepatocytes cultured for 2 h without fibronectin. n/d, not detected.

quent to the action of focal adhesion tyrosine kinases. Although these tyrosine kinases are inhibited by genistein it would have been useful, in retrospect, to have determined the effects of suramin and lovastatin on the fibronectin mediated induction of ribonuclease activity. However, a ribonuclease seems the most likely candidate to mediate the loss of CYP2C11 and CuZnSOD mRNAs, especially in view of the generalized loss of poly(A⁺) mRNA (Fig. 6a), as well as the inhibitory effect of genistein on both ribonuclease activity and CYP2C11 loss produced by fibronectin (Fig. 6b). Finally, this "spread but not degraded" population also is composed of hepatocytes treated with inhibitors of RNA transcription and translation, which also prevent the fibronectin-mediated induction of ribonuclease activity (data not shown).

In contrast to the fibronectin-mediated loss of CYP2C11, CuZnSOD, and poly(A⁺) mRNAs the results presented in Fig. 7a show that the abundance of albumin mRNA remains relatively constant throughout a 24-h culture period. Similarly, incubation of extracts of hepatocytes treated with fibronectin mimic the culture situation by enhanced degradation of in vitro transcribed CYP2C11 mRNA but not of albumin mRNA (Fig. 7b). The resistance of in vitro transcribed albumin mRNA to degradation seems not to be caused by protection by proteins present in the hepatocyte extracts, because the addition of bovine pancreatic ribonuclease A resulted in a complete degradation of this transcript. Thus it remains to be determined whether the selectivity between CYP2C11 and albumin mRNAs resides in the specificity of the ribonuclease induced by fibronectin.

In conclusion, the experimental system described here provides a unique opportunity to investigate further the relationships between cell shape, ribonuclease induction, and the specificity controlling mRNA degradation. From an applied view, greater consideration of hepatocyte shape and function may improve the utility of this in vitro system in pharmacological and toxicological research.

References

- Beindl W, Mitterauer T, Hohenegger M, Ijzerman AP, Nanoff C and Freissmuth M (1996) Inhibition of receptor/G protein coupling by suramin analogues. *Mol Pharmacol* **50**:415–423.
- Blaauboer BJ and Paine AJ (1979) Attachment of rat hepatocytes to plastic substrata in the absence of serum requires protein synthesis. *Biochem Biophys Res Commun* **90**:368–374.
- Bleasdale JE, Thakur NR, Gremban RS, Bundy GL, Fitzpatrick FA, Smith RJ and Bunting S (1990) Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. *J Pharmacol Exp Ther* **255**:756–768.
- Clayton DF, Harrelson AL and Darnell JE (1985) Dependence of liver-specific transcription on tissue organisation. *Mol Cell Biol* **5**:2623–2632.
- Cuthbert JA and Lipecky PE (1997) Regulation of proliferation and Ras localization in transformed cells by products of mevalonate metabolism. *Cancer Res* **57**:3498–3505.
- Davidoff AN and Mendelow BV (1992) Puromycin is a potent and specific inhibitor of tyrosine kinase activity in HL-60 cells. *Anticancer Res* **12**:1761–1766.
- Davis W and Schultz RM (1997) Role of the first round of DNA replication in reprogramming gene expression in the preimplantation mouse embryo. *Mol Reprod Dev* **47**:430–434.
- Dougall WC and Nick HS (1991) Manganese superoxide dismutase: A hepatic acute phase protein regulated by interleukin-6 and glucocorticoids. *Endocrinology* **129**:2376–2384.
- Folkman J and Moscona A (1978) Role of cell shape in growth control. *Nature (Lond)* **273**:345–349.
- Gazit A, Oshero N, Posner I, Yaish P, Poradosu E, Gilon C and Levitzki A (1991) Tryphostins 2: Heterocyclic and α -substituted benzylidenemalononitrile tryphostins as potent inhibitors of EGF receptor and ErbB2/neu tyrosine kinases. *J Med Chem* **34**:1896–1907.
- Gonzalez FJ (1989) The molecular biology of cytochrome P450s. *Pharmacol Rev* **40**:243–288.
- Hashimoto Y and Soderling TR (1987) Calcium-calmodulin-dependent protein kinase II and calcium-phospholipid protein kinase activities in rat tissues assayed with a synthetic peptide. *Arch Biochem Biophys* **252**:418–425.
- Hewlett EL, Sauee KT, Myers GA, Cowell JL and Guerrant RL (1983) Induction of a novel morphological response in chinese hamster ovary cells by pertussis toxin. *Infect Immunol* **40**:1198–1203.
- Lenneras H and Fager G (1997) Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clin Pharmacokinet* **32**:403–425.
- Maeda Y, Nunomura K and Ohtsubo E (1990) Differential scanning calorimetric study of the effect of intercalators and other kinds of DNA-binding drugs on the stepwise melting of plasmid DNA. *J Mol Biol* **215**:321–329.
- Morgan ET and Gustafsson J-A (1987) Sex specific isozymes of cytochrome P450. *Steroids* **4**:5213–245.
- Morgan ET, MacGeoch C and Gustafsson J-A (1985a) Hormonal and developmental regulation of expression of the hepatic microsomal steroid 16 α hydroxylase cytochrome P450 apoprotein in the rat. *J Biol Chem* **260**:11895–11898.
- Morgan ET, MacGeoch C and Gustafsson J-A (1985b) Sexual differentiation of cytochrome P450 in rat liver: Evidence for a constitutive isozyme as the male specific 16 α hydroxylase. *Mol Pharmacol* **27**:471–479.
- Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW (1996) P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6**:1–42.
- Niwa T, Koide N, Tsuji T, Imaoka S, Ishibashi F, Funae Y and Katagiri M (1996) Cytochrome P450s of isolated hepatocytes in spheroid and monolayer cultures. *Res Commun Mol Pathol Pharmacol* **91**:372–378.
- Obrig TG, Culp WJ, McKeehan WL and Hardesty B (1971) The mechanism by which cycloheximide and related glutarimide antibiotics inhibit peptide synthesis. *J Biol Chem* **246**:174–181.
- Odenthal M, Neubauer K, Baralle FE, Peters H, Meyer zum Buschenfelde KH and Ramadori G (1992) Rat hepatocytes in primary culture synthesise and secrete cellular fibronectin. *Exp Cell Res* **203**:289–296.
- Paine AJ (1990) The maintenance of cytochrome P450 in rat hepatocyte culture: Some applications of liver cell cultures to the study of drug metabolism, toxicity and the induction of the P450 system. *Chem-Biol Interact* **74**:1–31.
- Paine AJ (1995) Heterogeneity of cytochrome P450 and its toxicological significance. *Hum Exp Toxicol* **14**:1–7.
- Paine AJ (2000) Constitutive expression of cytochromes P450 in hepatocyte culture, in *Hepatocyte Review* (Berry MN and Edwards AM eds) Kluwer, Dordrecht, The Netherlands, in press.
- Peterson G (1995) Evaluation of the biochemical targets of genistein in tumour cells. *J Nutr* **125** (Suppl):784S–789S.
- Powis G, Bonjouklian R, Berggren MM, Gallegos A, Abraham R, Ashendel C, Zalkow L, Matter WF, Dodge J, Grindey G et al. (1994) Wortmannin, a potent and selective inhibitor of phosphatidylinositol-3-kinase. *Cancer Res* **54**:2419–2423.
- Rana B, Mischoulon D, Xie Y, Bucher NL and Farmer SR (1994) Cell-extracellular matrix interactions can regulate the switch between growth and differentiation in rat hepatocytes: Reciprocal expression of C/EBP α and immediate-early response transcription factors. *Mol Cell Biol* **14**:5858–5869.
- Runge D, Runge DM, Bowen WC, Locker L and Michalopoulos GK (1997) Matrix induced re-differentiation of cultured hepatocytes and changes of CCAAT/enhancer binding proteins. *J Biol Chem* **272**:873–881.
- Ruoslahti E (1988) Fibronectin and its receptors. *Annu Rev Biochem* **57**:375–413.
- Seglen PO, Berg TO, Blankson H, Fengsrud M, Hølen I and Stromhaug PE. (1996) Structural aspects of autophagy. *Adv Exp Med Biol* **389**:103–111.
- Schuetz EG, Li D, Omiecinski CJ, Muller-Eberhard U, Kleinman HK, Elswick B and Guzelian PS (1988) Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. *J Cell Physiol* **134**:309–323.
- Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E, Loriolle F et al. (1991) The bisindolylmaleimide GF-109203X is a potent and selective inhibitor of protein kinase C. *J Biol Chem* **266**:15771–15781.
- Wang X-J, Hodgkinson CP, Wright MC and Paine AJ (1997) Temperature-sensitive mRNA degradation is an early event in hepatocyte de-differentiation. *Biochem J* **328**:937–944.
- Wright MC, Wang X-J, Pimenta M, Ribeiro V, Paine AJ and Lechner MC (1996) Glucocorticoid receptor independent transcriptional induction of cytochrome P450 3A1 and its potentiation by a glucocorticoid. *Mol Pharmacol* **50**:856–863.

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