Increased Sp1-dependent Transactivation of the LAMγ1 Promoter in Hepatic Stellate Cells Co-cultured with HepG2 Cells Overexpressing Cytochrome P450 2E1*

Received for publication, July 8, 2002, and in revised form, December 17, 2002
Published, JBC Papers in Press, January 15, 2003, DOI 10.1074/jbc.M206790200

Natalia Nieto‡ and Arthur I. Cederbaum
From the Department of Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, New York, New York 10029

Laminin is a basement-membrane protein that increases in liver fibrosis. To study the role of oxidative stress on laminin expression, hepatic stellate cells (HSC) were co-cultured with HepG2 cells that do or do not express (E47 or C34 cells, respectively) CYP2E1, a potent generator of oxygen radicals. Co-incubation of HSC with E47 cells increased laminin β1 and γ1 proteins compared with co-incubation with C34 cells; this increase was prevented by antioxidants and CYP2E1 inhibitors. Similar results were observed in co-culture with primary hepatocytes from saline- or pyrazole-treated (with high levels of CYP2E1) rats. Laminin α1 chain was not detectable in the HSC in any of the systems; however, laminin α2 chain increased in HSC co-cultured with E47 cells. Synthesis but not turnover of laminin β1 and γ1 proteins was increased in HSC in the E47 co-culture. Laminin β1 and γ1 mRNAs were up-regulated in HSC in the E47 co-culture because of transcriptional activation of both genes. Transfection experiments in HSC with reporter constructs driven by the laminin γ1 promoter showed maximal responsiveness with the −230/+106 and the −1400/+106 constructs in the E47 system. Gel-shift assays demonstrated an increase in Sp1 binding to the laminin γ1 promoter in HSC when co-incubated with E47 cells, which was blocked by an anti-Sp1 antibody. Co-transfection of a Sp1 expression vector further increased the responsiveness of the −330LAMγ1-CAT reporter vector in HSC in the HSC/E47 system. These results show that diffusible CYP2E1-derived oxidative stress mediators induce synthesis of laminins by a transcriptional mechanism in HSC. Such interactions between hepatocytes and HSC may be important during liver fibrosis.

The laminins are a family of adhesive glycoproteins composed of high molecular weight disulfide-bonded heterotrimers composed of αβγ chains. To date, five α, three β, and two γ chains have been described, forming at least 12 trimeric laminin isoforms (1). Although laminins are distinguished by both subunit composition and tissue distribution, the overall domain structure is well conserved (1). Laminin 1 (EHS laminin) is an 820-kDa heterotrimer consisting of one each of α1 (400-kDa), β1 (220-kDa), and γ1 (200-kDa) chains, and laminin 2 (Merosin) is an 800-kDa heterotrimer formed of α2 (380-kDa), β1 (220-kDa), and γ1 (200-kDa) chains. Both covalent and noncovalent interactions contribute to the basic structure of most laminins, a cruciform with three short arms and one long arm (2). The individual laminin subunits share a common structural motif composed of repeated epidermal growth factor-like domains interrupted by globular domains.

A number of biological activities have been attributed to laminin, including cell attachment, differentiation, and migration, along with interactions with other matrix components (3). Laminin has also been implicated in the process of tumor invasion and metastasis (4). Different isoforms of the laminin molecule may vary with respect to tissue distribution and developmental expression. The β1 and the γ1 chains are expressed in most tissues that produce basement membranes, but their ratios vary considerably (5).

In the liver, increased deposition of laminins, the main non-collagenous glycoproteins in all basement membranes, was demonstrated in rats with chemically induced carcinoma (6) and within the lobule of livers from patients with malignancies (7). In contrast, in normal adult livers, laminins are located mainly in the portal tracts and are only sparsely deposited in the space of Disse (8). In human liver, the laminin isoforms have not been clearly identified so far, and only information from rodents is available. Rat hepatic stellate cells (HSC),1 the main source of extracellular matrix components in both normal and fibrotic livers, express β1, γ1, and an α chain of 380 kDa that probably corresponds to unprocessed form of the α2 chain (9). Normal adult hepatocytes do not express laminin 1 in vivo, but synthesize both β1 and γ1 chain mRNAs after a few hours in culture (6, 7). Several hepatoma cell lines of either human or rat origin express both β1 and γ1 chains at high levels and a 380-kDa polypeptide that is genetically different from the α1 chain but stained by polyclonal anti-laminin antibodies (10). Although most studies have focused on laminins polymerized into a basement membrane, much laminin is found freely soluble and diffusible. Furthermore, although the polymerization of laminin in vitro occurs by a self-assembly mechanism, basement membranes are formed at discrete sites close to the cell membrane (11).

The 5′-flanking region of the mouse laminin γ1 (LAMγ1) gene has been cloned and characterized (12). The LAMγ1 gene

---

1 The abbreviations used are: HSC, hepatic stellate cells; CYP2E1, cytochrome P450 2E1; ROS, reactive oxygen species; MEM, minimal essential medium; CAT, chloramphenicol acetyltransferase.
appears to contain two transcription start sites (−169 and −234) and it does not contain a TATA or CAAT box, however, it has several interesting features, including the presence of ten GC boxes, which act as putative binding sites for the redox-sensitive transcription factor Sp1 (two in tandem and the rest monomeric), and a stretch of nine nearly identical repeats of 11 nucleotides between −200 and −450 with the sequence 5′-CCG/GT/CCCG/AT/CCT-3′. The consensus sequence for cAMP responsiveness is also present in the LAM-1 promoter and is similarly found in the promoters of other extracellular-matrix proteins (12). These motifs act as transcription activators in several extracellular-matrix genes. The integrity of the CTC-rich region is required to promote LAMy1 activity. Consequently, it could be hypothesized that there is interplay between CTC and GC elements, GC boxes being predominant in the activation of truncated CTC-less LAMy1 promoter. The region from −830 to −224 appears to contain a negative regulatory element, which decreases the promoter activity of the LAM-1 gene. Deletion of −94 to −61 nucleotides reduced the promoter activity by severalfold in HepG2 cells, and deletion of 20 bp from −41 to −21 completely abolished the promoter activity in HepG2 cells (13). On the other hand, the LAMy1 promoter had a relatively high level of activity in NIH-3T3 cells, which synthesize little laminin. This result suggests that the 830-bp promoter segment may lack a negative regulatory element, which inhibits transcription in certain cells (12).

Ethanol and several other low molecular weight agents induce cytochrome P450 2E1 (CYP2E1), which is of special interest because it metabolizes and activates hepatic toxins as well as carcinogens and fatty acids (14). CYP2E1 is a loosely coupled enzyme that generates high amounts of reactive oxygen species (ROS) such as superoxide radical and H₂O₂ (15, 16). Oxidative stress plays an important role in mechanisms by which ethanol damages the liver (17). Our laboratory has carried out studies to evaluate whether hepatocytes with high levels of CYP2E1 can interact with nonparenchymal liver cells such as HSC via release of diffusible mediators (18, 19). In the present study we evaluated whether CYP2E1-derived oxidative stress could modulate the expression of basement-membrane proteins such as laminin, which are elevated during liver fibrosis, and the role of oxidative stress in this inductive up-regulation of laminin.

MATERIALS AND METHODS

Cell Culture—The model used in most of the experiments described below is based on the co-culture of HepG2 cell lines that express E47 cells or do not express C34 cells (the human CYP2E1 (20, 21) with primary HSC or with an immortalized rat stellate cell line (HSC-T6). Primary HSC were isolated from male Sprague-Dawley rats (600–750 g) (Charles River Breeding Laboratories, MA) by in situ liver perfusion with bacterial collagenase and pronase, followed by density-gradient centrifugation with Nycodenz according to published protocols (22). Results of Figs. 2–5 were with the primary HSC whereas other results were carried out with the HSC-T6 cells. Cell viability (95%) was assessed by the trypan blue exclusion method. Purity of the HSC fraction (97%) was determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previously (23). To extend results with the HepG2 cells to intact primary hepatocytes, selected experiments (Fig. 4) were carried out using hepatocytes isolated from saline control rats or from pyrazole-treated rats whose CYP2E1 levels (97%) were determined as described previous...
medium were transferred onto the plates containing the HSC, fresh medium was also added to the HSC that were plated with an empty insert as a reference group (not to be co-cultured with Hgp2 cells). 22 h later, the media from the complete co-culture systems were replaced with methionine-cysteine-free MEM plus 10% dialyzed fetal bovine serum, the cells were incubated for 2 h, after which they were pulse-labeled with 150 μCi of EasyTag™ EXPRESS™ S-S Protein Labeling Mix (PerkinElmer Life Sciences) for 0, 2, 4, 8, and 12 h to study the synthesis of laminin β1 and γ1. A set of samples was pulsed for 2 h in the presence of 40 μM cycloheximide, an inhibitor of protein synthesis, as a control for the analysis of laminin β1 and γ1 synthesis. The cells were washed in 1× phosphate-buffered saline and lysed at the indicated time points with 150 μl of 10 mM Tris-HCl buffer, pH 7.4, 0.5% Triton X-100, 1 mg/ml DNAse A, 150 mM NaCl, 0.5% sodium deoxycholate, 1% SDS, and 1 mM phenylmethylsulfonyl fluoride.

To assay the turnover of laminin β1 and γ1, defined as the loss of [35S]methionine-labeled intracellular laminin β1 and γ1 when HSC-T6 cells were chased with cold methionine, plus secretion of [35S]methionine-labeled laminin β1 and γ1 into the medium, the co-cultures were treated as above but pulse-labeled with the EXPRESS™ S-S mix for 24 h. The cells were then washed three times and chased with complete MEM supplemented with 300 μg/ml of cold methionine. Cells were washed in 1× phosphate-buffered saline and lysed at 0, 1, 2, 4, 8, and 12 h in the same lysis buffer. In all cases, laminin β1 and γ1 was immunoprecipitated with anti-laminin 1 IgG-protein G-agarose as follows: 40 μl of protein G-agarose was preincubated with 10 μl of preimmune rabbit serum for 15 min, followed by the addition of 50 μl of a 50% (v/v) suspension of protein G-agarose. After centrifugation for 2 min at 13,000 rpm, the supernatant was incubated with anti-laminin 1 IgG by rocking overnight at 4°C followed by addition of 50 μl of a suspension of protein G-agarose. Samples were centrifuged for 1 min at 13,000 rpm, the pellets were washed three times with lysis buffer, once with lysis buffer plus 2% SDS, and three times with 0.1 M Tris-HCl buffer, pH 6.8. Laminin β1 and γ1 were eluted by boiling for 5 min at 13,000 rpm, samples were centrifuged for 2 min to remove the protein G-agarose, resolved on a 5% SDS-PAGE, and dried. The intensity of the radioactive signal was quantified using PhosphorImager (Molecular Dynamics) and the ImageQuant software.

To evaluate total protein synthesis by HSC, cells were treated as above and incubated with the EasyTag™ EXPRESS™ S-S labeling mix for 0, 2, 4, 8, and 12 h, followed by addition of 30% trichloroacetic acid to stop the reaction. The cells were washed in 1× phosphate-buffered saline, and the trichloroacetic acid-precipitable counts were determined in a scintillation counter after resuspension of the pellets in scintillation liquid. To evaluate loss of [35S]methionine labeled HSC total protein, the co-cultures were pulse-labeled for 24 h as above, followed by chasing for 0, 1, 2, 4, 8, and 12 h. The HSC were treated with 30% trichloroacetic acid and washed, and the trichloroacetic acid-precipitable material was determined as described above.

Quantitative comparison of the intensity of the signal scanned in the PhosphorImager was performed using ImageQuant software.

**Results**

**Laminin β1, γ1, and α2 Protein Levels Increase in Primary HSC Co-cultured with E47 Cells**—Primary HSC were co-cultured with either the C34 or E47 cells. HSC lysates and aliquots of the incubation medium were collected at 1, 2, 3, 4, and 5 d and analyzed by Western blot for the expression of different laminin chains. A time-dependent increase in laminin β1 (220 kDa) and γ1 (200 kDa) production was observed in both systems but was higher in the E47 co-culture when compared with the C34 co-culture (Fig. 2A). At 3 d of co-culture there was a 2- to 4-fold increase in the HSC content of laminin β1 and γ1 chains, as well as a 3-fold increase in secretion of laminin β1 and γ1 to the medium of the E47 co-culture as assessed by Western blot (Fig. 2, B and C). β-Tubulin levels, as a control for loading, were identical in both co-cultures. This difference in the amount of laminin β1 and γ1 subunits in the culture medium in the E47 cell co-culture did not come from laminin β1 and γ1 produced by the E47 cells because the medium from C34 and E47 cells cultured alone did not show any significant differences in laminin β1 and γ1 content (Fig. 2D). High molecular weight bands in the 400-kDa region where laminin α1 would appear could not be detected in any of the blots in Fig. 2, B–D.

When the same samples were blotted and incubated with anti-laminin α2 chain antibody, laminin α2 chain expression was observed, and higher expression of laminin α2 was de-
tected intracellularly at 3 d in HSC co-cultured with E47 cells. Primary HSC were co-cultured with either C34 or E47 cells for 1, 2, 3, 4, and 5 d and cell lysates collected and analyzed by Western blot for laminin β1 and γ1 expression (A). A representative Western blot of HSC lysates (B) and culture medium (C) collected at 3 d is shown. Incubation medium from C34 and E47 cells cultured alone did not show any differences in laminin β1 and γ1 secreted (D). Arbitrary units under the blots refer to the intensity of the laminin β1 and γ1/β-tubulin ratio or the laminin β1 and γ1/fibrinogen ratio.

Fig. 3. Expression of laminin α2 chain in the co-cultures. Western blot showing the expression of laminin α2 chain in HSC co-cultured for 3 d with either C34 or E47 cells. A, intracellular expression of laminin α2 chain. B, levels of the α2 chain secreted into the incubation medium. C and D, Western blot of HSC lysates under non-denaturing conditions. HSC lysates after 3 d (C) and 5 d (D) from the co-cultures were electrophoresed under non-denaturing conditions and incubated with anti-laminin 1 antibody. The same blots were incubated for β-tubulin as a loading control. Arbitrary units under the blots refer to the ratio of laminin/β-tubulin. For D, arbitrary units refer to the ratio of the 800-kDa band/β-tubulin.

Fig. 2. Laminin β1 and γ1 protein levels increase in primary HSC co-cultured with E47 cells. Primary HSC were co-cultured with either C34 or E47 cells for 1, 2, 3, 4, and 5 d and cell lysates collected and analyzed by Western blot for laminin β1 and γ1 expression (A). A representative Western blot of HSC lysates (B) and culture medium (C) collected at 3 d is shown. Incubation medium from C34 and E47 cells cultured alone did not show any differences in laminin β1 and γ1 secreted (D). Arbitrary units under the blots refer to the intensity of the laminin β1 and γ1/β-tubulin ratio or the laminin β1 and γ1/fibrinogen ratio.

ROS Mediate the Induction of Laminin β1 and γ1 in Primary HSC Co-cultured with E47 Cells—To determine whether the increase by co-culturing HSC with E47 cells on laminin β1 and γ1 subunits appears to be delayed with respect to the increase in levels of expression of the individual α2, β1, and γ1 laminin chains or the 400-kDa dimer/s. The laminin heterotrimer is believed to play the major role in laminin’s action as a basement-membrane protein. Whether the presence of extracellular non-assembled α2, β1, γ1 chains has any physiological role is not known.

Laminin and Oxidative Stress
Fig. 4. Antioxidants and CYP2E1 inhibitors prevent the induction of laminin β1 and γ1 in the E47 co-culture. Primary HSC were incubated with C34 or E47 cells in the presence or absence of 2000 units of catalase or 50 μM vitamin E for 24 h, HSC were lysed, and laminin β1 and γ1 expression was analyzed by Western blot (A). Both systems were treated with the CYP2E1 inhibitors 5 mM diallylsulfide (DAS), 2 mM 4-methyl pyrazole (4MP), 0.1 mM sodium diethyldithiocarbamate (DETC), or 10 μM phenylisothiocyanate (PITC). HSC were also transfected with plasmids containing the cDNA for CYP2E1 in the sense and antisense orientation. Laminin β1 and γ1 expression in HSC lysates was evaluated by Western blot analysis. Arbitrary units under the blots refer to the intensity of the laminin β1 and γ1/β-tubulin ratio.

Hepatocytes from Pyrazole-treated Rats Increase Laminin β1 and γ1 Proteins by HSC but Do Not Affect the Incorporation of [35S]Methionine into Laminin. (A and B) The re-incorporation of newly synthesized laminin β1 and γ1—The results described above indicate that co-culture of HSC with E47 cells increases laminin β1 and γ1 protein levels. This effect could involve transcriptional activation of the LAMβ1 and LAMγ1 genes with elevated mRNA synthesis and/or mRNA stability, increased translational efficiency, or decreased turnover of newly synthesized laminin β1 and γ1 protein, with subsequent accumulation in the HSC. To address these possibilities, direct analysis of laminin β1 and γ1 protein synthesis, turnover, and mRNA levels by the co-cultures was performed. For the subsequent experiments, because of the need for large amounts of HSC and because transfection experiments with reporter constructs were to be used, a HSC-T6 cell line with a high efficiency for transfection was used rather than primary HSC.

Laminin β1 and γ1 protein synthesis was assessed by labeling with [35S]methionine for varying times, up to 12 h, followed by immunoprecipitation with anti-laminin 1 IgG as described under “Material and Methods.” There was a strong increase in the incorporation of [35S]methionine into laminin β1 and γ1 in the HSC/E47 co-culture compared with HSC cultured alone or in the presence of C34 cells (Fig. 6, A and B). Cycloheximide blocked laminin β1 and γ1 synthesis in all systems, validating that the increase in the laminins β1 and γ1 [35S]methionine signal was caused by a protein synthesis-dependent reaction. Total protein synthesis by HSC was not altered by co-culturing with E47 cells (Fig. 6C). These results suggest that an increase in laminin β1 and γ1 synthesis is associated with the induction of saline- and pyrazole-hepatocytes, respectively, when compared with HSC cultured alone (Fig. 5, basal conditions, lanes marked as “−”). This increase by both co-cultures was prevented by added catalase and vitamin E, indicating the involvement of ROS. In addition, the increase in laminin β1 and γ1 proteins by both co-cultures was decreased by CYP2E1 inhibitors: 5 mM diallylsulfide, 0.1 mM sodium diethyldithiocarbamate, and 10 μM phenylisothiocyanate (Fig. 5). These results suggest that CYP2E1-derived ROS contribute to the increase in laminin β1 and γ1 protein content of HSC in the saline-hepatocyte/HSC co-culture, and to the further increase produced by the pyrazole-hepatocytes/HSC co-culture.

CYP2E1-derived Diffusible Mediators Increase the Synthesis of Laminin β1 and γ1 Proteins by HSC but Do Not Affect the Turnover of Newly Synthesized Laminin β1 and γ1—The incorporation of [35S]methionine into laminin β1 and γ1 was performed in the HepG2 cell lines with primary hepatocytes, freshly isolated primary HSC were co-incubated with primary hepatocytes from either control or pyrazole-treated rats. Pyrazole induces CYP2E1 protein expression in hepatocytes about 3- to 4-fold over the levels present in saline control hepatocytes and stabilizes the protein against degradation (24). Laminin β1 and γ1 protein levels increased 3-fold and more than 6-fold in HSC cultured with saline- and pyrazole-hepatocytes, respectively, when compared with HSC cultured alone (Fig. 5, basal conditions, lanes marked as “−”). This increase by both co-cultures was prevented by added catalase and vitamin E, indicating the involvement of ROS. In addition, the increase in laminin β1 and γ1 proteins by both co-cultures was decreased by CYP2E1 inhibitors: 5 mM diallylsulfide, 0.1 mM sodium diethyldithiocarbamate, and 10 μM phenylisothiocyanate (Fig. 5). These results suggest that CYP2E1-derived ROS contribute to the increase in laminin β1 and γ1 protein content of HSC in the saline-hepatocyte/HSC co-culture, and to the further increase produced by the pyrazole-hepatocytes/HSC co-culture.
of both proteins in the presence of CYP2E1-mediated oxidative stress. Although synthesis of the laminin α2 chain was not determined in these experiments, Western blot analysis confirmed that laminin α2 chain levels were elevated after 12 h of culture with the HSC/E47 co-culture (Fig. 6D), analogous to the increase found with primary HSC co-cultured with E47 cells (Fig. 3A).

To study the apparent turnover of laminin β1 and γ1 chains, the HSC were first labeled with [35S]methionine for 24 h and, after washing, chased with excess unlabeled methionine for up to 12 h. Laminins β1 and γ1 proteins were immunoprecipitated at time points of 0, 1, 2, 4, 8, and 12 h, and the remaining intracellular incorporated label quantified by SDS-polyacrylamide gel electrophoresis and fluorography. The decrease in total intracellularly labeled protein was determined by trichloroacetic acid precipitation, washing, and counting the solubilized HSC pellet for [35S]methionine in trichloroacetic acid-precipitable protein. The apparent turnover of the newly
synthesized laminin β1 and γ1 subunits and total proteins was calculated from the semilogarithmic plot of counts incorporated per minute versus time. Prior to the initiation of the chase (0 h), there was an increase in [35S]methionine-labeled laminin β1 and γ1 in the HSC/E47 co-culture (as described above) (Fig. 7, A and B). Pulse-chase experiments revealed that the turnover of newly synthesized laminin β1 and γ1 (reflected as time for 50% loss of intracellular [35S]methionine counts) was 5.6 h for stellate cells cultured alone, 5.7 h for stellate cells co-incubated with C34 cells, and 6.0 h for stellate cells co-cultured with E47 cells (Fig. 7, A and B).

Loss of intracellular radioactivity incorporated into laminin from the HSC labeled with methionine could reflect turnover of the labeled laminin β1 and γ1 proteins because of intracellular degradation or secretion into the medium or both. To evaluate the latter, samples of media from the pulse-chase experiment were collected at the same time points, and immunoprecipitation of laminins β1 and γ1 chains was carried out as described above (Fig. 8). Very low rates of secretion of the newly synthesized laminin were observed over the 12-h chase under these conditions. In fact, 25 times more protein had to be immunoprecipitated to observe the bands shown in Fig. 8A than for the results of Fig. 7A. The loss of intracellular cpm as a function of time cannot be accounted for by the small secretion of newly synthesized laminin β1 and γ1 during the 12-h chase as the increase in total medium cpm was just a few percent of the decline in total cellular cpm. The amount of laminin secreted to the culture medium was minimal in both co-culture systems. Yurchenco et al. (11) expressed the β1, β2, and γ1 chains subunits of laminin 1 in all combinations in a near-null background, and showed that in the absence of its normal partners, the β1 chain is secreted as intact protein and protein that had been cleaved in the coiled-coil domain. In contrast, the β2 and γ1 chains, expressed separately or together, remain intracellular with formation of β2γ1 or β2γ2, but not γ1γ1, disulfide-linked dimers. Secretion of the β and γ chains required simultaneous expression of all three chains and their assembly into αβγ heterotrimers. They concluded that the α chain can be delivered to the extracellular environment as a single subunit, whereas the β and γ chains cannot, and that the α chain drives the secretion of the trimeric molecule. Such an α chain-dependent mechanism could allow for the regulation of laminin export into a nascent basement membrane, and might serve an important role in controlling basement-membrane formation. As mentioned above, the laminin α1 chain was not observed in the HSC, whereas the α2 chain was detected both in cell lysates...
and in the culture medium, suggesting that the laminin β1 and γ1 subunits into the medium. Importantly, the experiments described above suggest that turnover of laminin β1 and γ1 proteins was similar in both co-culture systems and not likely to explain the increase in laminin β1 and γ1 proteins by the HSC/E47 co-culture. Turnover of total HSC proteins were also similar (about 3.6 to 3.9 h) for HSC cultured alone, or co-incubated with C34 or E47 cells (Fig. 6C). There were no differences in the secretion of total proteins in both co-culture systems (Fig. 7C).

**Induction of Laminin β1 and γ1 Proteins in the E47 Co-culture Involves Transcriptional Regulation**—To determine why laminin β1 and γ1 synthesis was elevated, total RNA was isolated from HSC-T6 cultured alone or with C34 or E47 cells and was analyzed by Northern blot for laminin α1, β1, and γ1 chains mRNAs. Laminin α1 mRNA was not detected in any of the cell models. The HSC/C34 co-culture resulted in an increase in laminin β1, but not γ1 mRNA levels over mRNA levels in the HSC cultured alone (Fig. 9A). The HSC/E47 co-culture produced an increase in laminin γ1 mRNA as well as further induction of laminin β1 mRNA. Overall, there was a 2- to 3-fold increase in β1 and γ1 laminin mRNA levels in HSC co-cultured with E47 cells compared with the HSC/C34 co-culture (Fig. 9A). Nuclear in vitro transcription assays were performed to study whether the CYP2E1-mediated effect on laminin β1 and γ1 mRNA levels in HSC is regulated at the transcriptional level. As shown in Fig. 9B, enhanced laminin β1 and γ1 expression occurs through a transcriptional mechanism with both co-culture systems, however, the newly transcribed laminin β1 and γ1 mRNAs were increased in HSC co-incubated with E47 cells when compared with HSC co-incubated with C34 cells. It is not understood why laminin γ1 transcription appears to be up-regulated in the HSC/C34 co-culture compared with HSC alone, whereas the mRNA levels are similar.

**Identification of the Sequences of the LAMγ1 Promoter in HSC Required for CYP2E1-mediated Responsiveness**—Transient-transfection experiments in HSC-T6 with chimeric constructs harboring progressive 5’ deletions of the LAMγ1 promoter linked to the CAT reporter gene were performed to identify the regions of the LAMγ1 promoter required for CYP2E1-dependent activation. HSC cells were transfected with the constructs shown in Fig. 10. The percentage of acetylation of chloramphenicol in the HSC co-cultured with E47 cells and transfected with the −330LAMγ1-CAT and the −1400LAMγ1-CAT was significantly higher (about 46-fold) than in HSC cultured alone or with C34 cells (Fig. 10, A and B). These data are consistent with previous findings (Fig. 9, A and B) that CYP2E1-
most similar (about 45% acetylation of chloramphenicol). On were normalized to account for the slight increase in the loading con-

were increased in both the GAPDH and S14 mRNAs were used as housekeep-

of newly transcribed GAPDH and S14 mRNAs were used as housekeeping genes. There was a slight increase in both the GAPDH and S14

mRNA from HSC cultured alone or with C34 or E47 cells. The signals

involves transcriptional regulation. Interestingly, the activity of the

LAM\gamma1 promoter is exerted, at least in part, at the

transcriptional level. Interestingly, the activity of the

−330LAM\gamma1-CAT and −1400LAM\gamma1-CAT constructs were very similar (about 45% acetylation of chloramphenicol). On the other hand, the activity of the −580LAM\gamma1-CAT (and −2500LAM\gamma1-CAT) constructs was significantly lower (4% of acetylation of chloramphenicol) and very close to the basal levels of promoter activity by the shorter constructs (−200LAM\gamma1-CAT and −2500LAM\gamma1-CAT). These data suggest that the −230 to −480 region (and perhaps the −1400 to −2500 region) of the LAM\gamma1 gene may contain a silencer-like element which reduces promoter activity. Reporter activity was similar for HSC incubated alone compared with the HSC/C34 co-culture for the various constructs, which is in agreement with the similar laminin \gamma1 mRNA levels found in the HSC compared with the HSC/C34 co-culture (Fig. 9A). In general, the pattern of promoter expression was similar for all three systems (HSC alone, HSC/C34, and HSC/E47 co-culture), i.e. all three systems showed positive response to the −330LAM\gamma1-

CAT and −1400LAM\gamma1-CAT constructs, and negative responsiveness to the −580LAM\gamma1-CAT and −2500LAM\gamma1-CAT constructs. However, the E47 co-culture clearly showed the most robust responses to the −330LAM\gamma1-CAT and −1400LAM\gamma1-CAT constructs, likely a reflection of the presence of redox-sensitive sites in these regions. Thus, the promoter responses are not unique for CYP2E1 effects but are enhanced by CYP2E1-derived diffusible factors.

CYP2E1-derived Oxidative Stress Transactivates the LAM\gamma1 Promoter in HSC Co-incubated with E47 Cells through a Sp1-dependent Mechanism—The LAM\gamma1 promoter possesses several putative binding sites for redox-sensitive transcription factors including Sp1, AP-1, and NFκB (30). To determine whether any of these transcription factors could, in response to the increase in ROS produced by the E47 co-culture, transac-
vivate the LAM\gamma1 promoter, electrophoretic mobility-shift as-
says were carried out with nuclear extracts from HSC incubated alone or cultured with either C34 or E47 cells (Fig. 11A and inset). HSC co-cultured with E47 cells showed increased binding of Sp1 to an oligonucleotide containing its putative binding site GGGCGG when compared with HSC cultured alone or with C34 cells. There were no changes in the binding activity of NFκB and AP-1 (The inset shows an electrophoretic mobility-shift assay loading only 0.5 μg of protein from the same samples). Competition studies with a 1000-fold excess of cold Sp1 oligonucleotide blunted the binding of the radiolabeled Sp1 oligonucleotide. The complex of Sp1 protein-DNA was sup-
pershifted with an anti-Sp1 antibody, demonstrating specific-
ity of the complex (Fig. 11A, last lane). Southwestern analysis carried out with a double-stranded oligonucleotide obtained by PCR amplification of the −230 to −150 region of the LAM\gamma1 promoter (where strong reporter activity was noted, Fig. 10) and nuclear proteins from HSC cultured alone, with C34 cells, or with E47 cells, showed a single band of about 100 kDa, which corresponds to the molecular mass of Sp1 (95–106 kDa) for all samples (Fig. 11B). The binding activity was comparable be-

between the HSC and the HSC/C34 co-culture but was 2-fold higher in HSC incubated with E47 cells. The increase in bind-
ing activity for the E47 co-culture was prevented by the CYP2E1 inhibitor diallylsulfide and by the free-radical-scav-

enging agent tempol (Fig. 11B). To verify that the band was indeed Sp1, a Southwestern analysis was carried out in which the membrane was incubated with an excess of anti-Sp1 anti-
body before hybridization with the double-stranded DNA oligo-

nucleotide. No signal was obtained (data not shown), indicating that the detected band was Sp1, and suggesting that the pro-
tein mediating the CYP2E1 effects on the LAM\gamma1 promoter may be Sp1. To eliminate the possibility that increased binding and transactivation of the LAM\gamma1 promoter could be caused by increased levels of Sp1 in the HSC co-cultured with E47 cells, a Western blot analysis of total Sp1 protein was carried out with nuclear protein extracts. Results in Fig. 11C show that no differences in Sp1 protein content were observed in HSC cul-
tured alone or with C34 or E47 cells. Finally, co-transfection of HSC with an Sp1 expression vector plus the −330LAM\gamma1-CAT reporter construct, which contains seven putative binding sites for Sp1 and whose binding activity was induced about 8-fold in the HSC/E47 co-culture compared with the C34 system (Fig. 10B), were carried out (Fig. 11D). Although the co-transfection with the Sp1 expression vector enhanced reporter activity in all three systems (HSC alone, HSC/C34, and HSC/E47), HSC transfected with the Sp1 expression vector and co-cultured with E47 cells showed higher CAT activity for the −330LAM\gamma1-CAT reporter construct than did HSC cultured alone or with C34 cells (about 5-fold) (Fig. 11D). A Western blot analysis was also carried out to validate that Sp1 levels were elevated after transfection and levels were comparable in the three HSC systems (Fig. 11D, lower panel).

DISCUSSION

Basement membranes are cell-associated heteropolymers that are essential for tissue development and maintenance. The functions of these extracellular matrices are both architec-
tural and informational, with basement membranes acting as substrata, filters, and solid-phase agonists (31–33). In the liver, laminin is present during maturation of the organ and accumulates in the adult during the capillarization process that occurs in alcoholic cirrhosis and hepatocarcinogenesis (8, 34). Laminin γ1 mRNA is abundant in HSC, the major site of matrix formation, and it is also expressed at high levels in transformed hepatoma cell lines and during chemically induced hepatocarcinogenesis in the rat (6, 35), but it is not present in normal hepatocytes (8). The presence of laminin γ1 chain is a prerequisite for basement-membrane formation, with its absence causing an early embryonic lethality (36). Besides self-assembling, laminins also interact with other laminin isoforms as well as with nonlaminin extracellular matrix molecules such as perlecan, nidogen, and collagen to form a polymeric matrix; such associations appear necessary for proper basement membrane assembly (1).

Previous work in our laboratory has been aimed at characterizing the intercellular communication between the hepatocyte and the stellate cell to better understand the mechanisms by which oxidative stress and other mediator molecules perpetuate the fibrogenic response in stellate cells (18, 19). A coculture model containing HSC with a HepG2 cell line that overexpresses cytochrome P450 2E1 (E47 cells) or a control HepG2 cell line (C34 cells) was developed. The interest in CYP2E1 was due to its activation of numerous hepatotoxins, generation of oxidative stress, and possible role in contributing to alcohol-induced liver injury (14, 17, 37–42). This co-culture system, with constant generation of ROS, revealed increased translation of collagen mRNA by a ROS-dependent mechanism. Because of its importance as an abundant extracellular matrix component whose levels are elevated during liver injury, we extended our co-culture studies to the possible regulation of laminin production under oxidative stress conditions generated by CYP2E1.

Kleinman et al. (43) have shown that normal adult liver contains both laminin β1 and γ1 chains but lacks the α1 chain. Initial results shown in Figs. 2 and 3 revealed a time-dependent increase in both intra- and extracellular laminin β1, γ1, and α2 proteins (after 3 days of culture) which was enhanced in HSC co-cultured with E47 cells compared with the HSC/C34 co-culture. The laminin α1 chain could not be detected, confirming its lack in normal liver (43). To show interactions between the β1, γ1, and α2 laminin chains, Western blots of

**FIG. 10. Identification of the minimal sequences of the LAMγ1 promoter in HSC required for CYP2E1-mediated responsiveness.** Transient co-transfection experiments of HSC with chimeric constructs harboring progressive 5′ deletions of the LAMγ1 promoter linked to the CAT reporter gene and with the null-RL luciferase vector were performed in HSC cultured alone or with C34 or E47 cells. A, representative blot for the chloramphenicol acetyltransferase reaction and thin-layer chromatography. B, schematic representation of the chimeric constructs together with the percentage of acetylation of chloramphenicol corrected for protein concentration and by transfection efficiency. ***, p < 0.001, compared with basal expression. ●●●, p < 0.001, compared with HSC cultured alone or with C34 cells.
HSC lysates collected after 3 or 5 d of co-culture were carried out under nondenaturing conditions. The laminin 1 antibody used recognizes the α1/H9251, β1/H9252, and γ1/H9253 chains of laminin. Two bands of about 400 and 800 kDa were found after 5 d of culture, whereas the 400-kDa band was found after 3 d of culture. The 400-kDa band may likely be the dimers β1/γ1, β1/β1, and/or γ1/γ1, because the molecular mass of the β1 chain is about 220 kDa and that of the γ1 chain is about 200 kDa. The 800-kDa band may likely be the heterotrimer α2β1γ1 because the molecular mass of the α2 chain under native conditions would be of about 380 kDa plus 220 kDa of the β1 and 200 kDa of the γ1. Although other α chains (α3, α4, and α5) have been described, none of them have been detected in the liver except for the α2 chain (9). Of note is the fact that although there seems to be a time-dependent increase in laminins α2, β1, and γ1 with higher levels of expression in the E47 co-culture, the potential assembly of the three subunits to the 800-kDa heterotrimer is delayed, relative to the increase in levels of the individual chains, or to the assembly into dimers of β1 and γ1 (400 kDa).

The amount of H2O2 and lipid peroxidation end products in HSC or in the medium was previously shown to be increased by the E47 cell co-culture (18, 19). The antioxidant defense in HSC did not change with any of the culture conditions (data not shown). Evidence for oxidative stress involvement in the increase in laminin β1 and γ1 proteins by the E47 co-culture is based on the prevention of these effects by addition of antioxidants such as catalase or vitamin E to the incubation medium and by CYP2E1 inhibitors such as diallylsulfide, 4-methyl pyr-
azole, sodium diethylthiocarbamate, and phenylisothiocyanate. Transfection of E47 cells with an antisense CYP2E1 construct lowered laminin β1 and γ1 protein expression to basal levels, whereas transfection with a sense CYP2E1 plasmid into E47 or C34 cells further increased laminin β1 and γ1 expression. The relevance of these findings was further extended to co-cultures of HSC with primary hepatocytes from pyrazole-treated rats, with high CYP2E1 content, when compared with co-cultures with hepatocytes from saline-treated rats.

Experiments were carried out to determine the mechanism(s) responsible for the increase in laminin β1 and γ1 levels by the E47 co-culture. The synthesis of laminin β1 and γ1 chains was similar between HSC cultured alone or with C34 cells, but an increase in synthesis was found in HSC cultured with E47 cells. This effect was blocked by cycloheximide. The turnover of laminin β1 and γ1 proteins was similar in HSC cultured alone or with C34 or E47 cells, and the export of laminin β1 and γ1 chains to the culture medium was very low during the 12-h chase; thus, changes in laminin β1 and γ1 degradation and/or secretion do not account for the increase in laminin β1 and γ1 proteins. These results indicate that increased synthesis of laminin protein is one mechanism of regulation for the induction of laminin β1 and γ1 protein by the E47 co-culture. We next analyzed whether elevated mRNA levels could account for the increase in synthesis of laminin protein found in the E47 system. Northern blot analysis revealed elevated laminin β1 and γ1 mRNAs in the E47 compared with the C34 co-culture. Nuclear run-on experiments documented increased synthesis of both mRNAs. Thus, enhanced laminin β1 and γ1 expression in the E47 system results from transcriptional activation of the LAMB1 and LAMγ1 genes. There is specificity in the ability of CYP2E1-derived mediators to interact with the HSC and stimulate the synthesis of certain proteins, e.g. collagen (19) and laminin, whereas total protein synthesis is not altered, nor is the synthesis of other HSC proteins such as catalase, tissue inhibitor of metalloproteinase 1, or metalloproteinase 13 altered (19).

Consistent with these results, transient transfection of HSC co-cultured with E47 cells with chimeric constructs driven by different sequences of the LAMγ1 promoter indicated the presence of two redox-sensitive enhancer elements located in the −230 to −150 and −1300 to −480 regions that were not stimulated in HSC cultured alone or with C34 cells. Little data are available on the molecular mechanisms involved in the transcriptional regulation of basement-membrane genes in liver fibrosis. Laminin is highly expressed in hepatic fibrosis (44). The 5'-untranslated region of LAMγ1 contains a stem-loop structure spanning from +76 to +106. Deletion of 47 bp within the 5'-untranslated region (+59 to +106) of the LAMγ1 completely blocked promoter activity in astrocytes, confirming that this downstream region could be one of the major points of transcriptional regulation (30). The chimeric constructs used for transient-transfection studies in the HSC systems contained the 5'-untranslated region of the first exon, which is GC-rich and has a stem loop structure; whether these could be redox-sensitive and operate coordinately with other factors released by the E47 system is not known.

The regulation of the expression of laminin γ1 mRNA in hepatoma cells involves several regions within the 2-kb promoter, and transfection of LAMγ1 promoter fragments in these cells indicated that regulatory elements are located between −594 and −94 bp (35). The −230 to −150 region of the promoter contains several monomeric Sp1-binding sites and a cAMP-responsive element. The LAMγ1 gene promoter contains multiple cognate sites for Sp1 binding which have the ability to recruit other transcription factors to initiate transcription from TATA-less promoters (45). TATA-less promoters typically have multiple transcription-initiation sites that are located very close or within the regions that contain Sp1-binding sites. The multiple Sp1-binding sites in these classes of genes suggest that they could be redox-sensitive promoters. Overexpression of Sp1 in normal hepatocytes increases endogenous LAMγ1 gene expression and co-transfected LAMγ1 promoter (46). High-binding activity was observed in Sp1-transfected nuclear extracts. Sp1 and laminin γ1 mRNA are both highly expressed in human hepatocarcinomas, particularly at the invasive front (47).

In view of the above, we evaluated Sp1-binding activity in the different HSC systems. Electrophoretic mobility-shift assays showed increased Sp1-binding activity in nuclear extracts from HSC co-incubated with E47 cells compared with that of HSC cultured alone or with C34 cells. The DNA-protein complex was shifted by an anti-Sp1 antibody and competed by a 1000-fold excess of cold oligonucleotide containing the Sp1-binding site. Two other well known redox-sensitive transcription factors, AP-1 and NFκB, showed the same binding activity in all three systems. The AP-1 site located at −650 bp is not involved in LAMγ1 transcription in hepatoma cells, whereas several sequences in the −480 bp to −175 bp region have been identified and may bind specific regulatory factors, including Sp1 and immediate early gene products coded by the cAMP-family (48). The results described above suggest that the increased promoter activity in transient-transfection studies with the −230 to −150 reporter construct could be mediated by increased binding of the redox-sensitive transcription factor Sp1 to this region.

To verify this, we performed Southwestern analysis to determine the binding capacity of the −230 to −150 region of the promoter to nuclear proteins from HSC cultured alone or with C34 or E47 cells. This region of the promoter binds to a protein of about 100 kDa, and the binding is increased 2- to 3-fold in HSC co-cultured with E47 cells. A role for a CYP2E1-mediated effect and for ROS was validated by addition of diallylsulfide, a CYP2E1 inhibitor, and of 4-hydroxy-tempo (tempol), a wide-spectrum free radical scavenger, both of which were able to prevent the enhanced Sp1 binding of the E47 co-culture. Sp1 is a dimer of molecular masses 95 and 106 kDa. To determine whether this binding protein was Sp1, the same samples were analyzed by Southwestern blot incubating the membrane with anti-Sp1 antibody before hybridization with the −230 to −150 double-stranded oligonucleotide; no binding was detected after incubation with the antibody. To ensure that the increase in Sp1 binding observed was not a result of increased Sp1 synthesis, a Western blot analysis was carried out with nuclear proteins from HSC cultured alone or with C34 or E47 cells. This region of the promoter binds to a protein of about 100 kDa, and the binding is increased 2- to 3-fold in HSC co-cultured with E47 cells. A role for a CYP2E1-mediated effect and for ROS was validated by addition of diallylsulfide, a CYP2E1 inhibitor, and of 4-hydroxy-tempo (tempol), a wide-spectrum free radical scavenger, both of which were able to prevent the enhanced Sp1 binding of the E47 co-culture. Sp1 is a dimer of molecular masses 95 and 106 kDa. To determine whether this binding protein was Sp1, the same samples were analyzed by Southwestern blot incubating the membrane with anti-Sp1 antibody before hybridization with the −230 to −150 double-stranded oligonucleotide; no binding was detected after incubation with the antibody. To ensure that the increase in Sp1 binding observed was not a result of increased Sp1 synthesis, a Western blot analysis was carried out with nuclear proteins from HSC cultured alone or with C34 or E47 cells, but no differences were observed among the three systems. These results suggest that the increased LAMγ1 promoter activity found with the HSC/E47 co-culture transfected with the −230 to −150 construct could be due to increased Sp1 binding. Furthermore, in experiments in which a Sp1 expression vector was co-transfected along with the −330LAMγ1-CAT reporter construct, a 5-fold increase in CAT activity was detected in the HSC/E47 co-culture compared with HSC cultured alone or with C34 cells.

The LAMγ1 gene is transcriptionally up-regulated by interleukin-1β due to an increased binding of NFκB to a κB consensus sequence on the LAMγ1 promoter (49). Both interleukin-1β and TGF-β transiently increase laminin γ1 mRNA due to enhanced binding of nuclear proteins on the GC-rich bcn-1 motif in the promoter. The cooperative induction of the LAMγ1 promoter and the endogenous LAMγ1 gene by TFE3 and Smad3 is
Laminin and Oxidative Stress

augmented by the TGF-β signaling pathway (50). In our reporter assays, there was decreased activity in all systems (HSC alone, HSC/C34, and especially HSC/E47 co-culture) with the

−2500LAMγ1-CAT construct, suggesting the presence of a si-

encer-like element between the −1300 and −2400 region. Not

much is known about the sequence of the LAMγ1 promoter

upstream of −1000 bp. We have not analyzed possible redox-
sensitive elements further upstream that could be responsible

for the transactivation of the −1400 to −480 region of the pro-
moter. Whether this could be mediated directly by ROS or

involve other factors such as cytokines, most of which are

redox-sensitive molecules, still remains to be elucidated.

In summary, these results suggest that CYP2E1, present in

the hepatocyte, can release diffusible mediators, most likely

stable ROS such as H₂O₂ and lipid peroxidation metabolites,

the hepatocyte, can release diffusible mediators, most likely

redox-sensitive molecules, still remains to be elucidated.

Acknowledgments—We thank Dr. Yoshiko Yamada (National

Institutes of Health) for providing the LAMγ1-CAT constructs and Dr.

Robert Tjian (University of California, Berkeley) for the Sp1

expression vector.

REFERENCES

1. Tunggal, P., Smyth, N., Paulsson, M., and Ott, M. C. (2000) Microsc. Res. Tech. 51, 214–227

2. Sasaki, M., Kleinman, H. K., Huber, H., Deutzmann, R., and Yamada, Y. (1988) J. Biol. Chem. 263, 16536–16544

3. Timpl, R., Rohde, H., Robey, P. G., Rennard, S. I., Foidart, J. M., and Martin, G. R. (1979) J. Biol. Chem. 254, 9933–9937

4. Liotta, L. A., Rao, C. N., and Barsky, S. H. (1983) J. Biol. Chem. 258, 21475–21480

5. Boot-Handford, R. P., Kurkinen, M., and Prockop, D. J. (1987) Nucleic Acids Res. 15, 1–11

6. Tunggal, P., Smyth, N., Paulsson, M., and Ott, M. C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 15189–15194

7. Kedar, Y., Freese, E., and Hempel, F. (1997) Mol. Brain. Res. 47, 87–98

8. Koop, D. R. (1992) FASEB J. 6, 724–730

9. Ekstrom, G., and Ingelman-Sundberg, M. (1989) Biochem. Pharmacol. 38, 1313–1319

10. Gersky, L. D., Koop, D. R., and Coon, M. J. (1984) J. Biol. Chem. 259, 6812–6817

11. Tsukamoto, H., and Lu, S. C. (2001) FASEB J. 15, 1335–1349

12. Tsukamoto, H., Park, S. L., and Cederbaum, A. I. (2002) Hepatology 35, 62–73

13. Gersky, L. D., Koop, D. R., and Coon, M. J. (1984) J. Biol. Chem. 259, 6812–6817

14. Rescan, P. Y., Clement, B., Yamada, Y., Segui-Real, B., Guguen-Guillouzo, C., and Guillouzo, A. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 10384–10388

15. Tunggal, P., Smyth, N., Paulsson, M., and Ott, M. C. (2000) J. Biol. Chem. 275, 20136–20145

16. Kedar, Y., Freese, E., and Hempel, F. (1997) Mol. Brain. Res. 47, 87–98

17. Koop, D. R. (1992) FASEB J. 6, 724–730

18. Mitchell, P. J., and Tjian, R. (1989) Cell 69, 519–527

19. Singh, H., Lebowitz, J. H., Baldwin, A. S., and Sharp, P. A. (1988) Cell 52, 415–425

20. O’Neill, B. C., Suzuki, H., Loomis, W. P., Denissenko, O., and Bomsztyk, K. (1997) Am. J. Pathol. 149, 1411–1420

21. Vidal, M., and Edgar, D. (1999) J. Biol. Chem. 274, 17063–17069

22. Engvall, E., and Wewer, U. M. (1996) Nature 382, 362–363

23. Mitchell, P. J., and Tjian, R. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 337–341

24. Wu, D., and Cederbaum, A. I. (1993) J. Biol. Chem. 268, 21486–21493

25. Nieto, N., Friedman, S. L., and Cederbaum, A. I. (1999) Pharmacol. Rev. 51, 1–78

26. Nieto, N., Friedman, S. L., and Cederbaum, A. I. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9922–9926

27. Singh, H., Lebowitz, J. H., Baldwin, A. S., and Sharp, P. A. (1988) Cell 52, 415–425

28. Baffet, G., Loreal, O., and Campion, J. P. (1997) Virchows. Arch. A Pathol. Anat. Histopathol. 431, 87–93

29. Clemens, B., Rescan, P. Y., Baffet, G., Loreal, O., Lifshitz, D., Campion, J. P., and Guguen-Guillouzo, C. (1988) Hepatology 8, 794–801

30. Mahler, J. J., and Tager, A. (1994) Hepatology 19, 764–770

31. Clemons, B., Rescan, P. Y., Clement, B., Yamada, Y., Guguen-Guillouzo, C., and Guillouzo, A. (1990) J. Biol. Chem. 265, 423, 197–205

32. Wu, D., and Cederbaum, A. I. (1993) J. Biol. Chem. 268, 21486–21493

33. Kleinman, H. K., Ehnhara, I., Killen, P. D., Sasaki, M., Cannon, B. F., Yamada, Y., and Martin, G. R. (1987) Dev. Biol. 122, 373–378

34. Reuben, R., Shah, A., Biempiu, L., Zern, M. A., and Caja, M. J. (1992) Matrix 12, 36–43

35. Malet, S. T., and Baltimore, D. (1989) Cell 57, 103–113

36. Mitchell, P. J., and Tjian, R. (1989) Science 245, 371–378

37. Lietard, J., Musso, O., Theret, N., L’Helguetach, A., Campion, J. P., Yamada, Y., and Clement, B. (1997) Am. J. Pathol. 151, 1663–1672

38. Christey, B., and Nathans, D. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8737–8741

39. Richardson, C. A., Gordon, K. L., Couer, W. G., and Bomsztyk, K. (1995) Am. J. Physiol. 268, F273–F278

40. Kato, Y., Suzuki, H., Higaki, Y., Denissenko, O., Schullery, D., Abrass, C., and Bomsztyk K. (2002) J. Biol. Chem. 277, 11375–11384
Increased Sp1-dependent Transactivation of the LAMγ1 Promoter in Hepatic Stellate Cells Co-cultured with HepG2 Cells Overexpressing Cytochrome P450 2E1
Natalia Nieto and Arthur I. Cederbaum

J. Biol. Chem. 2003, 278:15360-15372.
doi: 10.1074/jbc.M206790200 originally published online January 15, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M206790200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 49 references, 15 of which can be accessed free at
http://www.jbc.org/content/278/17/15360.full.html#ref-list-1