Cellular sources of glutathione S-transferase P in primary cultured rat hepatocytes: localization by *in situ* hybridization

Sue J. LEE,*†, Scott L. FRIEDMAN†, Richard WHALEN* and Thomas D. BOYER*

*Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322, U.S.A. and †Liver Center, University of California, San Francisco, CA 94110, U.S.A.

Hepatocytes *in vivo* express Alpha and Mu but not Pi forms of glutathione S-transferase (GST). GST P (a fetal Pi form) appears in rat hepatocytes after 2 days in primary culture, which suggests that hepatocytes may undergo dedifferentiation [Abramovitz, Ishigaki and Listowsky (1989) *Hepatology* 9, 235–239]. However, in this and other studies, primary rat hepatocyte cultures were shown by immunohistochemistry to contain significant numbers of lipocytes (Ito cells). Freshly isolated lipocytes contained GST activity when assayed with chlorodinitrobenzene (680 nmol/min per mg), and expression of Alpha, Mu and Pi forms of GST was detected by Western-blot analysis. Expression of GST P persisted during culture of the lipocytes. *In situ* hybridization of the cultured cells was performed to define whether hepatocytes, lipocytes or both expressed the enzyme. Lipocytes in culture

contained abundant GST P transcripts. Hepatocytes contained no GST P transcripts after 12 h in culture, and after 24 h, only a few hepatocytes expressed this enzyme. After 48 h in culture all hepatocytes contained GST P transcripts, and the number of transcripts continued to increase up until 72 h. Therefore, in freshly isolated preparations of hepatocytes and early in hepatocyte culture, measurable levels of GST P protein or message appeared to reflect the presence of lipocytes. After 48 h in culture almost all of the GST P reflected expression by the hepatocytes. Lipocytes constitutively expressed Alpha-, Mu- and Pi-class GSTs and had significant intracellular levels of GSH (5.2 nmol/mg of protein). Lipocytes are capable therefore of detoxifying a number of injurious compounds.

INTRODUCTION

The glutathione S-transferases (GSTs) are a family of dimeric proteins that play important roles in both the intracellular transport of hydrophobic molecules and the metabolism of toxic compounds [1–9]. The cytosolic GSTs can be grouped into at least four different families termed Alpha, Mu, Pi and Theta based on N-terminal sequences, substrate specificities and affinity for non-substrate ligands [10,11]. *In vivo*, the levels of some of the isoforms can be increased by treatment with drugs such as phenobarbital or antioxidants which increase rates of transcription [12–14]. Levels of GSTs in the liver also change during development, with disappearance of the Pi-class isoenzyme and appearance of Alpha- and Mu-class enzymes preceding parturition [15]. In primary culture, GST expression by rat hepatocytes changes in a manner that suggests dedifferentiation of the hepatocytes. Adult hepatocytes *in vivo* lack GST P and contain large amounts of the Alpha- and Mu-class GSTs. After 24 h in culture, expression of GST P by hepatocytes is reported to increase whereas levels of Alpha- and Mu-class GST decrease or remain unchanged [16–18].

Interpretation of the published culture data is based on the assumption that hepatocyte cultures are virtually pure. However, it has recently been recognized that early primary hepatocyte cultures (i.e. within 48 h) contain up to 10% lipocytes [19]. The unrecognized presence of the vitamin A-storing non-parenchymal cells led to a misbelief that hepatocytes synthesized collagen [20,21]. Subsequent studies demonstrated that it was lipocytes and not hepatocytes that accounted for the production of collagen during primary culture of rat hepatocytes [19]. Because some hepatic non-parenchymal cells express certain forms of GST [22], we have reassessed the cellular sources of GST P in primary cultures of rat hepatocytes. Specifically, we have determined

which forms of GST are expressed by lipocytes, and then localized expression of GST P during culture by *in situ* hybridization.

MATERIALS AND METHODS

Materials

Arabinogalactan was obtained from Larex International, Tacoma, WA, U.S.A. Polyclonal rabbit anti-(chicken desmin) was from Dako Corp., Santa Barbara, CA, U.S.A., and rabbit anti-[rat GST Ya (1), Yb₁ (3) and Yp (7)] (nomenclature for rat GSTs suggested by Jakoby et al. [23]) were purchased from Bioprep, Dublin, Ireland. Restriction enzymes were from Gibco-BRL, Grand Island, NY, U.S.A. [³²P]dCTP and [³⁵S]thioUTP, megaprime labelling kit and enhanced chemiluminescence kit were from Amersham, Arlington Heights, IL, U.S.A. T3 and T7 RNA polymerases and transcription kit were from Promega, Madison, WI, U.S.A. Pronase and collagenase B were purchased from Boehringer-Mannheim Biochemicals, Indianapolis, IN, U.S.A. All other chemicals were of reagent grade and were purchased from Sigma Chemical Corp., St. Louis, MO, U.S.A.

Hepatocyte and lipocyte cultures

Hepatocytes were isolated from male Sprague–Dawley rats by collagenase perfusion and centrifugal elutriation as described previously [24]. Lipocytes were isolated by *in situ* perfusion with collagenase and Pronase followed by density-gradient centrifugation with arabinogalactan [25]. Only cells from the top layer of the gradient were used and were more than 99% pure lipocytes [25]. Hepatocytes and lipocytes were plated at a density of 1×10^6 – 2×10^6 cells/ml on rat tail collagen-coated 35- or 100-mm-diam. plastic plates or 2-well plastic slides for immunohistochemistry and *in situ* hybridization. Cells were plated in

Medium 199 [26] containing 20% (v/v) serum [10% (v/v) calf and 10% (v/v) horse], which was changed every 24 h. Viability of hepatocytes and purity of lipocytes was determined as described previously [24].

Western blot and measurement of enzyme activity

Hepatocytes and lipocytes were harvested into cold PBS (10 mM sodium/potassium phosphate, pH 7.4, 150 mM NaCl) homogenized on ice and centrifuged at approx. 150 000 *g* (45 000 rev./min) for 30 min at 4 °C in a 70.1 Ti rotor. The cytosolic fraction was collected, frozen and kept at -20 °C until used. Protein concentration was determined using the Bio-Rad protein assay with BSA as the standard. For the Western-blot analysis, each lane contained either 1 µg of the purified standard GST (YaYa, Yb₁Yb₁, or YpYp) or 30 µg of lipocyte cytosolic protein. Electrophoresis was performed using a 13.5% running and 4% stacking polyacrylamide gel. Proteins were electrotransferred to Immobilon-P poly(vinylidene difluoride) paper (Millipore, Bedford, MA, U.S.A.). The primary antibody was anti-Ya, anti-Yb or anti-Yp (1:2000). The proteins were identified using alkaline phosphatase-labelled anti-(rabbit IgG) antibodies (Bio-Rad Laboratories, Melville, NY, U.S.A.) or the enhanced chemiluminescence system (Amersham). The resultant radiograms from the latter were subjected to densitometry using an ISCO gel scanner, and the areas determined using a Digital computer.

Enzyme activity in lipocyte cytosol was determined spectrophotometrically using chlorodinitrobenzene, cumene hydroperoxide, *p*-nitrobenzyl chloride and ethacrynic acid as substrates [27]. All assays were performed in triplicate and are expressed as means ± S.D.

RNA preparation and Northern-blot hybridization

Total RNA was extracted from cultured cells by lysis in guanidine thiocyanate [28], and RNA concentration was estimated from *A*₂₆₀. Total RNA (15 µg) was denatured in 2.2 M formaldehyde/50% formamide and loaded on to each lane of a 1% (w/v) agarose gel containing 2.2 M formaldehyde. The quality of the RNA was judged by staining the gels with ethidium bromide. The RNA was transferred to a Nylon membrane (NEN Research Laboratory-Dupont, Boston, MA, U.S.A. in 20 × SSC (1 × SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0) overnight. The membrane was then baked in an 80 °C oven for 2 h and kept refrigerated until the time of hybridization.

The cDNA for GST Ya was provided by Dr. Cecil Pickett (Schering-Plough, Kenilworth, NJ, U.S.A.) [29], that for GST Yp by Dr. Barch (Northwestern University, Chicago, IL, U.S.A.) [30] and that for mouse albumin by Dr. Morgan (Emory University, Atlanta, GA, U.S.A.). A 0.55 kb fragment of the Ya cDNA and a 1 kb fragment of the mouse albumin DNA were purified from a *Pst*I digest of the respective plasmids. A 0.8 kb fragment of Yp cDNA was purified from an *Eco*RI-*Sal*I digest of the plasmid obtained from Dr. Barch. The cDNA fragments were labelled with [³²P]dCTP using the random primer labelling kit (Amersham). The blots were hybridized with the cDNAs at 65 °C for 8–16 h, washed three times for 20 min each in 1 × SSC at 65 °C and then exposed to Kodak film. The resultant radiograms were subjected to densitometric scanning as described previously [31].

Immunohistochemistry

Hepatocytes and lipocytes were washed twice with PBS to remove dead cells and fixed in cold methanol (-20 °C) for 10 min. All subsequent steps were performed at room tem-

perature. Slides were air-dried and blocked for 30 min with PBS containing 5% (v/v) goat serum, followed by two washes with PBS containing 1% (v/v) goat serum. Slides were then incubated in PBS with 2% (v/v) goat serum containing one of the following primary antibodies: Ya (1:250), Yp (1:125), rabbit anti-(rat IgG) (1:250), desmin (1:25). After being washed twice with PBS containing 1% (v/v) goat serum, cells were incubated for 30 min with a 1:250 dilution of donkey anti-(rabbit IgG) antibody labelled with Texas Red (Amersham) in PBS containing 2% (v/v) goat serum. After two brief washes with PBS, the slides were mounted with glycerol/PBS and photographed on Ektachrome 400 slide film using a Nikon Diaphot microscope equipped for epifluorescence.

In situ hybridization

Hepatocytes were grown in Lab-Tek culture slides from Baxter. At various time points, the culture medium was removed by aspiration and the upper chambers of the culture slides were removed leaving the lower silicone gasket in place. The slides were then rinsed in sterile PBS and the cells fixed in 4% (v/v) paraformaldehyde with 0.1 M sodium phosphate buffer, pH 7.4, for 10 min at 4 °C. The slides were kept in 70% (v/v) ethanol at 4 °C until used.

Riboprobes labelled with [³⁵S]UTP were made using the Promega transcription kit. The complete rat Yp cDNA [30] was subcloned into Bluescript II KS phagemid (Stratagene, La Jolla, CA, U.S.A.) at the *Eco*RI-*Sal*I site. The orientation of the cDNA was determined by sequencing the insert. The plasmid was linearized with *Eco*RI, and the antisense RNA probe transcribed using T3 RNA polymerase. The sense probe was made by linearizing the plasmid with *Sal*I and using T7 RNA polymerase. The fixed slides were removed from the alcohol, washed in 0.5 × SSC, incubated with proteinase K (1 µg/ml in 0.5 M NaCl/10 mM Tris, pH 8.0) and then 0.5 × SSC, all at room temperature. The slides were prehybridized in 50% formamide hybridization buffer at 42 °C for 1–3 h. Radiolabelled sense or antisense probe (approx. 5 × 10⁵ c.p.m.) and 50 µg of tRNA were incubated overnight on the slides at 55 °C. The slides were then washed in 2 × SSC, treated with RNAase A (20 µg/ml), and washed serially for 20 min each in 2 × SSC, 0.1 × SSC and 0.5 × SSC. The slides were dehydrated for 2 min each in 50%, 70% and 90% ethanol all containing 0.3 M ammonium acetate. They were then desiccated for 3–4 h and coated in the dark with Kodak NTB 2 nuclear emulsion diluted 1:1 with water at 42 °C and then left to dry for 2 h. The dried slides were exposed for 3 weeks and then developed and fixed. Finally, they were stained with haematoxylin and eosin and then examined and photographed [32,33].

Figure 1 Western-blot analysis of lipocyte cytosol

Purified GST YaYa, Yb₁Yb₁ or YpYp (1 µg) was used as standard (S) and 35 µg of lipocyte cytosolic protein (L) was applied in separate lanes. Anti-Ya, -Yb₁ and -Yp antibodies were used as described in the Materials and methods section.

RESULTS

Cytosol prepared from freshly isolated lipocytes contained Alpha, Mu and Pi forms of GST as shown by immunoblot (Figure 1). Enzyme activities in the lipocyte cytosol with the various substrates were: with chlorodinitrobenzene, 680 ± 40 nmol/min per mg; with ethacrynic acid, no measurable activity; with cumene hydroperoxide, 76 ± 7.0 nmol/min per mg; with *p*-nitrobenzyl chloride, 340 ± 50 nmol/min per mg. An homogenate of the lipocytes contained 5.2 ± 0.42 nmol of gluta-

Figure 2 Northern-blot analysis of RNA isolated from freshly isolated rat lipocytes and hepatocytes

Total RNA (5 or 15 µg) was loaded in each lane. Either a radiolabelled mouse albumin cDNA or rat Yp cDNA was then used to identify specific transcripts.

Figure 3 Change in GST mRNA and Yp protein levels during culture of rat hepatocytes

RNA (15 µg) from specified times in culture was loaded in each lane and probed with radiolabelled cDNA for Ya (a) and Yp (b). (c) Changes in Yp protein expression were examined at the same time points.

Figure 4 Identification of lipocytes in primary culture of rat hepatocytes

After 48 h in culture, immunohistochemistry using anti-desmin antibodies was performed as described in the Materials and methods section. L, Lipocytes.

Figure 5 *In situ* hybridization of rat lipocytes

A radiolabelled sense (a) or antisense (b) GST P riboprobe was added to the cells after 48 h in culture, and *in situ* hybridization was performed as described in the Materials and methods section.

thione/mg of total cellular protein. To determine whether the lipocyte cultures contained hepatocytes, a Northern-blot analysis using RNA from freshly isolated lipocytes and hepatocytes was

Figure 6 *In situ* hybridization of rat hepatocytes

Cells were probed after 12 h (a), 24 h (b), 48 h (c) and 72 h (d) in culture with sense (not shown) or antisense GST P riboprobes as described in the Materials and methods section.

performed (Figure 2). The lipocytes did not contain albumin transcripts, indicating that the GST in the lipocyte cultures reflects expression by lipocytes and not hepatocytes.

As reported previously [16], during culture, hepatocytes lost GST Ya and acquired GST P transcripts (Figure 3). No GST P transcripts were identifiable in the hepatocytes until 24 h in culture, whereas, by Western-blot analysis the protein appeared to be present at the earliest time point. The intensity of the signal for GST P by Western-blot analysis increased during culture (Figure 3).

Previous studies have assumed that the changes in GST expression during culture reflect changes in the hepatocytes. Hepatocyte cultures after 48 h, however, are known to contain up to 10% lipocytes [19]. Staining of the cultures with anti-desmin antibodies demonstrated that the cultures in the current study also contained significant numbers of lipocytes (Figure 4).

In order to define more precisely the cellular source of GST P in the hepatocyte culture, immunohistochemical staining using anti-Ya and anti-Yp antibodies was performed. The anti-Ya antibodies gave a diffuse staining to the hepatocytes. Lipocytes stained strongly positive for the Yp protein, whereas the hepatocytes were negative for the Yp protein after 24 and 48 h in culture. Hepatocytes showed diffuse staining after 72 h of culture (results not shown). Lipocytes after 48 h in culture contained large amounts of the Yp transcripts as shown by *in situ* hybridization (Figure 5). No expression of Yp by the hepatocytes could be identified after 12 h in culture (Figure 6a). Expression of Yp transcripts by hepatocytes became evident after 24 h in

culture (Figure 6b) and increased progressively for at least 72 h (Figures 6c and 6d).

DISCUSSION

A number of investigators have demonstrated that GST P is expressed by rat hepatocytes during culture but not in freshly isolated cells [16–18]. These authors have assumed that the increase in this enzyme represented *de novo* expression by hepatocytes and failed to account for the fact that primary cultures of rat hepatocytes contain significant numbers of lipocytes [19]. In this study, freshly isolated lipocytes were shown to constitutively express GST P and to maintain expression during culture. Therefore, in freshly isolated preparations of hepatocytes and early in hepatocyte cultures, measurable GST P protein or message reflects the presence of lipocytes. As time in culture increases beyond 24 h, hepatocytes begin to express GST P. After 48 h in culture, hepatocytes account for the majority of the increase in GST P transcripts, with the lipocytes contributing only a small percentage to the total.

The expression of GST P by the cultured hepatocytes initially is not uniform but instead is limited to a few cells (Figure 6b). With increasing time in culture, all the hepatocytes express the enzyme. The initial variability in expression is unexplained, but could reflect the lobular heterogeneity of GST expression [34] which may persist for a short time in culture.

Increased expression of GST P during culture appears to reflect an increase in transcription [35]. The GST P gene is known

to contain both enhancers and at least one silencer region in the 5' flanking sequence [36,37]. The increase in expression of GST P during culture is thought to be due in part to inactivation of this silencer region [38]. The silencer region is identical with an interleukin 6 (IL-6)-inducible *trans*-activator LAP/IL-6-DBP binding site [37]. Although the role of IL-6 in the regulation of expression of GST P has not been explored, hepatocytes are known to contain IL-6 receptors and to change the expression in culture of a number of acute-phase proteins after exposure to IL-6 [39]. It is reasonable to suggest therefore that the absence of IL-6 from the culture medium may account for at least some of the increased expression of GST P by the cultured hepatocytes.

Lipocytes are normally present in the sinusoids of the liver and can be identified by the presence of large intracellular droplets of vitamin A. In the current study, freshly isolated lipocytes were shown to contain members of three families of GST and to have significant intracellular levels of GSH. Lipocytes would be expected therefore to detoxify a number of injurious agents including products of lipid peroxidation. Lipocytes become activated after a variety of different types of liver injury. One possible activating event is lipid peroxidation, which is a common feature of liver injury [40,41]. The response of lipocytes to oxidative stress therefore may be an important factor in their activation. Once activated, lipocytes express types-I, -II and -IV collagen and are the primary source of collagen in the cirrhotic liver [42]. Thus an understanding of how lipocytes deal with oxidative stress may be important in understanding the fibrotic process.

In conclusion, we have shown that both hepatocytes and lipocytes express GST P. In lipocytes, expression is constitutive whereas in hepatocytes expression of this fetal enzyme is observed only after at least 24 h in culture. Although lipocytes are present in hepatocyte cultures, the use of *in situ* hybridization has established that hepatocytes account for the bulk of the increase in GST P expression that occurs during culture. As GST P is frequently expressed in transformed and neoplastic cells [43–46], studies using primary rat hepatocyte cultures should allow for a better understanding of how expression of this enzyme is regulated in normal and transformed cells. In addition, in early culture of hepatocytes (i.e. < 24 h) GST P expression is due largely to the presence of lipocytes. Studies utilizing early primary cultures of hepatocytes to examine regulation of GST P expression will need to account for the early dual expression of this protein.

We appreciate the skilled secretarial assistance of Ms. Pamela Allen. This work was supported in part by grants from the N.I.H. (GM-31555, DK-37340).

REFERENCES

- Mannervik, B. (1988) *CRC Crit. Rev. Biochem.* **23**, 281–334
- Pickett, C. D. and Lu, Y. H. (1989) *Annu. Rev. Biochem.* **58**, 743–764
- Chasseaud, L. F. (1979) *Adv. Cancer Res.* **29**, 175–274
- Boyer, T. D. (1989) *Hepatology* **9**, 486–496
- Tipping, E. and Ketterer, B. (1981) *Biochem. J.* **195**, 441–442
- Wolkoff, A. W., Goresky, C. A., Sellin, J., Gatmaitan, Z. and Arias, I. M. (1979) *Am. J. Physiol.* **236**, E638–E648
- Senjo, M., Ishibashi, T. and Imai, Y. (1985) *J. Biol. Chem.* **260**, 9191–9196
- Homma, H. and Listowsky, I. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7165–7169
- Boyer, T. D. and Olsen, E. (1991) *Biochem. Pharmacol.* **42**, 188–190
- Mannervik, B., Alin, P., Gluthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. and Jornvall, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7202–7206
- Meyer, D. J., Coles, B., Pembble, S. E., Gilmore, K. S., Fraser, G. M. and Ketterer, B. (1991) *Biochem. J.* **274**, 409–414
- Rushmore, T. H. and Pickett, C. B. (1990) *J. Biol. Chem.* **265**, 14648–14653
- Ding, G., Ding, V., Rodkey, J. A., Bennett, C. D., Lu, A. Y. H. and Pickett, C. B. (1986) *J. Biol. Chem.* **261**, 7952–7957
- Guthenberg, C., Morgenstern, R., DePierre, J. and Mannervik, B. (1980) *Biochim. Biophys. Acta* **631**, 1–10
- Tee, L. B. G., Gilmore, K. S., Meyer, D. J., Ketterer, B., Vandenberghe, Y. and Yeoh, G. C. T. (1992) *Biochem. J.* **282**, 209–218
- Abramovitz, M., Ishigaki, S. and Listowsky, I. (1989) *Hepatology* **9**, 235–239
- Vandenberghe, Y., Glaire, D., Meyer, D. J., Guillouzo, A. and Ketterer, B. (1988) *Biochem. Pharmacol.* **37**, 2482–2485
- Vandenberghe, Y., Tee, L., Morel, F., Rogiers, V., Guillouzo, A. and Yeoh, G. (1991) *FEBS Lett.* **284**, 103–108
- Maher, J. J., Bissell, M., Friedman, S. L. and Roll, F. J. (1988) *J. Clin. Invest.* **82**, 450–459
- Diegelmann, R. F. and Guzelian, P. S. (1983) *Science* **219**, 1343–1345
- Tseng, S. C. G., Smucker, E. A. and Stern, R. (1983) *Hepatology* **3**, 955–963
- Steinberg, P., Schramm, H., Schladt, L., Robertson, L. W., Thomas, H. and Oesch, F. (1989) *Biochem. J.* **264**, 737–744
- Jakoby, W. B., Ketterer, B. and Mannervik, B. (1984) *Biochem. Pharmacol.* **33**, 2539–2540
- Irving, M. G., Roll, F. J., Huang, S. and Bissell, D. M. (1984) *Gastroenterology* **87**, 1233–1247
- Friedman, S. L. (1993) in *Methods in Toxicology* (Tyson, C. A. and Frazier, J. M., eds.), pp. 292–310, Academic Press, Orlando
- Irving, M. G., Roll, F. J., Huang, S. and Bissell, D. M. (1984) *Gastroenterology* **87**, 1233–1247
- Boyer, T. D., Kenney, W. C. and Zakim, D. (1983) *Biochem. Pharmacol.* **32**, 1843–1850
- Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159
- Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G., Argenbright, L. and Lu, A. (1984) *J. Biol. Chem.* **259**, 5182–5188
- Sugoka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T. and Muramatsu, M. (1985) *Nucleic Acids Res.* **13**, 6049–6057
- Lee, S. J. and Boyer, T. D. (1993) *Biochem. J.* **293**, 137–142
- Wilcox, J. N., Smith, K. M., Schwartz, S. M. and Gordon, D. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2839–2843
- Wilcox, J. N., Smith, K. M., Williams, L. T., Schwartz, S. M. and Gordon, D. (1988) *J. Clin. Invest.* **82**, 1134–1143
- Redick, J. A., Jakoby, W. B. and Baron, J. (1982) *J. Biol. Chem.* **257**, 15200–15203
- Vandenberghe, Y., Morel, F., Foriers, A., Ketterer, B., Vercruysse, A., Guillouzo, A. and Rogiers, V. (1989) *FEBS Lett.* **251**, 59–64
- Sakai, M., Okuda, H. and Muramatsu, M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9456–9460
- Imagawa, M., Osada, S., Koyama, Y., Suzuki, T., Hiron, P. C., Diccianni, M. B., Morimura, S. and Muramatsu, M. (1991) *Biochem. Biophys. Res. Commun.* **179**, 293–300
- Morimura, S., Okuda, A., Sakai, M., Imagawa, M. and Muramatsu, M. (1992) *Cell Growth Differ.* **3**, 685–691
- Kishimoto, T., Akira, S. and Taga, T. (1992) *Science* **158**, 593–597
- Kamimura, S., Gaal, K., Britton, R. S., Bacon, B. R., Triadafilopoulos, G. and Tsukamoto, H. (1992) *Hepatology* **16**, 448–453
- Maher, J. J. and Friedman, S. L. (1993) *Semin. Liver Dis.* **13**, 13–20
- Maher, J. and McGuire, R. F. (1990) *J. Clin. Invest.* **86**, 1641–1648
- Kitahara, A., Satoh, K., Nishimura, K., Ishikawa, T., Ruike, K., Sato, K., Tsuda, H. and Ito, N. (1984) *Cancer Res.* **44**, 2698–2703
- Guthenberg, C., Warholm, M., Rane, A. and Mannervik, B. (1986) *Biochem. J.* **235**, 741–745
- Jensson, H., Eriksson, L. C. and Mannervik, B. (1985) *FEBS Lett.* **187**, 115–120
- Sato, K. (1989) *Adv. Cancer Res.* **52**, 205–255