Volume 313, number 2, 155–159 FEBS 11768 November 1992

Transcriptional- and post-transcriptional-dependent regulation of glutathione *S*-transferase expression in rat hepatocytes as a function of culture conditions

Yves Vandenberghe[a], Lisa Tee[b], Vera Rogiers[a] and George Yeoh[b]

[a]*Department of Toxicology, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium* and [b]*Department of Physiology, University of Western Australia, Nedlands 6009, Australia*

Received 18 September 1992

Transcriptional activity of the glutathione *S*-transferase (GST) α (subunits 1 and 2), μ (subunits 3 and 4) and π (subunit 7) gene families has been analyzed using the nuclear 'run-on' technique on adult rat hepatocytes maintained for 4 days in conventional culture and for 4 and 12 days in co-culture with rat liver epithelial cells. Several medium conditions are included in this study, namely with or without fetal calf serum and with nicotinamide or dimethylsulphoxide. Hepatocytes co-cultured for 4 days maintain approximately 30–70% of the α gene family transcriptional activity, whatever the medium conditions, when compared to freshly isolated hepatocytes. A marked decrease is observed after 12 days of co-culture or when hepatocytes are maintained in conventional culture. The transcriptional activity of the μ gene family is maintained at 40–160% when hepatocytes are cultured with or without fetal calf serum, and is inducible by nicotinamide (approximately 4-fold) and dimethylsulphoxide (approximately 2-fold) in conventional culture and/or in co-culture. In contrast to freshly isolated hepatocytes, GST π gene transcriptional activity is observed in conventional and co-cultured hepatocytes, irrespective of the medium conditions. Dimethylsulphoxide treatment however, represses the expression of GST 7 in vitro. These results demonstrate that the expression of GST α, μ and π genes in conventional and co-cultured rat hepatocytes is controlled primarily at the level of transcription. It cannot be excluded, however, that dimethylsulphoxide stabilizes the GST mRNA levels in vitro.

Glutathione *S*-transferase; Transcriptional activity; Rat; Hepatocyte; Culture

1. INTRODUCTION

The glutathione *S*-transferases (GSTs, EC 2.5.1.18) are a family of functional dimeric enzymes which not only catalyze the conjugation of reduced glutathione with a wide variety of electrophiles, including carcinogens, but also have important transport functions [1,2]. More recently, some isoenzymes have been described in terms of their capacity for reducing organic hydroperoxides [3], leukotriene C4 synthesis [4] and isomerization of, for example, Δ^5-3-ketosteroid [5].

The rat cytosolic GSTs are homo- and heterodimers of at least 11 different subunits [6], which, on the basis of present evidence involving DNA and amino acid sequences, enzymatic properties and immunological cross-reactivity, have been classified into four families: α, μ, π and θ [7,8]. In liver tissue, the major subunits are 1 and 2, and 3 and 4 which belong to the α and μ family, respectively [9]. Subunit 7, absent in adult liver parenchymal cells, has been found in fetal liver [10], appears at an early stage during hepatocarcinogenesis [11] and has recently been detected in cultured adult rat hepatocytes [12].

Adult rat hepatocytes, which are known to undergo differential changes when seeded in culture [13], have previously been used in vitro to study the expression of GST isoenzymes. It has been demonstrated that GST enzymatic activity [14], subunit composition [15], de novo synthesis of GST subunits in vitro [15] and steady-state mRNA levels, using complementary DNA sequences for GST subunits 1/2, 3/4 and 7 [16,17], vary depending on the culture and media conditions used.

It was observed that, as in vivo, GST subunits in vitro are regulated independently. Differential changes in the GST isoenzymes, during the culture period, can be summarized as a general loss of the α class of subunits, a maintenance or increase of the μ class of subunits and the de novo expression of subunit 7 in conventional culture, as well as in hepatocytes co-cultured with rat liver epithelial cells.

In the present study, adult rat hepatocytes were maintained under different culture and medium conditions in order to determine the level of regulation of the different gene families. Comparison with earlier studies [16,17] should identify whether any correlation exists between the changing mRNA levels coding for the GST

Correspondence address. Y. Vandenberghe, Department of Toxicology, Searle, European Development Center, Rue Granbompré 11, 1348 Mont-Saint-Guibert, Belgium. Fax: (32) (10) 450 290.

subunits 1/2, 3/4 and 7 and the transcriptional activity of the respective genes in cultured hepatocytes, or if the steady-state mRNA levels are the consequence of RNA stabilization. A nuclear 'run-on' experiment has been performed on nuclei isolated from conventionally and co-cultured adult rat hepatocytes, maintained in standard medium or medium containing 10% fetal calf serum (FCS), 25 mM nicotinamide (Nic) or 2% dimethylsulphoxide (DMSO).

2. MATERIALS AND METHODS

2.1. *Cell isolation and culture*

Hepatocytes from adult male Sprague–Dawley rats weighing 180–200 g were isolated by the two-step collagenase perfusion method as previously described [18]. The liver was first washed for 10 min with HEPES (*N*-2-hydroxy-ethylpiperazine-*N*2-ethane sulfonic acid) buffer, pH 7.6, and then for 20 min with a 0.025% collagenase solution buffered with HEPES. Cells were collected in Leibovitz-15 medium and seeded at a density of 10×10^6 cells per 175 cm flasks in 25 ml of medium containing 10% FCS. The standard medium (St) consisted of 75% minimal essential medium and 25% Medium 199, containing 200 μg/ml bovine albumin, 10 μg/ml bovine insulin and 10% FCS. The medium was first changed 4 h after cell seeding and every day thereafter. Co-cultures were set up by adding approximately 1.4×10^7 rat liver epithelial cells per flask in order to reach confluency within 24 h. Cultures which were not mixed with epithelial cells are termed 'conventional' cultures. 4 h after seeding, 7×10^{-5} M and 3.5×10^{-6} M hydrocortisone hemisuccinate was added to conventional cultures and co-cultures, respectively. These concentrations were maintained during the culture period. Four different media conditions were tested: St medium plus fetal calf serum (St+FCS); St medium minus fetal calf serum (St−FCS); St medium minus fetal calf serum containing 25 mM nicotinamide (St−FCS+Nic); 50% St medium and 50% Williams' E medium minus fetal calf serum with 2% dimethylsulphoxide (SW/W−FCS+DMSO).

2.2. *Run-on transcription in isolated nuclei and hybridization*

Nuclei were isolated from freshly isolated (50×10^6 hepatocytes) and cultured hepatocytes (5 flasks of 10×10^6 hepatocytes each/cultured period and culture medium) by the method of Becker [19], suspended in 50% glycerol, 5 mM $MnCl_2$, 1 mM $MgCl_2$, 5 mM DTT, 20 mM Tris, pH 7.4, frozen in liquid nitrogen and stored at −80°C. Transcription in isolated nuclei, isolation of ^{32}P-labelled RNA, and hybridization to nitrocellulose filters were performed as described previously [20] with some modifications. Briefly, isolated nuclei (5×10^6) were incubated in a reaction mixture consisting of 50 mM HEPES (pH 8.0), 150 mM NH_4Cl, 1 mg/ml nuclease-free BSA, 1 mM $MnCl_2$, 12.5% glycerol, 0.1 mg/ml heparin, 3.5 mM $MgCl_2$, 1 μl (60 U) RNasin, 0.25 mM DTT, 0.5 mM ATP, GTP, CTP and UTP, 100 μCi of [α-^{32}P]UTP for 20 min at 25°C. The mixture was adjusted to final concentrations of 5 mM Tris (pH 7.5), 5 mM $MgCl_2$, 5 mM $CaCl_2$ and 1 μl (1 U) RQ1 DNAse was added and the mixture incubated for 5 min at 37°C. After treatment with proteinase K (150 μg/ml) at 37°C for 30 min, RNA was isolated by phenol/chloroform extraction followed by ethanol precipitation in the presence of 0.05 mg/ml transfer RNA. After treatment again with RQ1 DNAse as above, RNA was isolated by phenol/chloroform precipitation followed by ethanol precipitation as above, and hybridized at 42°C for 72 h in 55% formamide, 4 × SSC, 0.1 M sodium phosphate (pH 6.8), 5 × Denhardt's solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 10% dextran sulphate to 0.25 pmol of each of the following nitrocellulose-bound DNA: (a) albumin genomic subclones B, C and D [21], (b) GST cDNA pGSTr 155 [22]; (c) GST cDNA JT9L; (d) GST cDNA pGSTr 7 [23]; (e) GAPDH cDNA pRGAPDH-13 [24]; (f) pUC 18; (g) pBR 322. Filters were washed at 65°C three times in 1 × SSC, 0.1% SDS and then twice in 0.1 × SSC, 0.1% SDS and associated radiolabel was visualized by fluorography and assessed by optical densitometry. Densitometry signals were converted to relative transcription rates by subtracting the background signal (pUC 18 or pBR 322) and correcting for the fraction of primary transcripts hybridizable to the recombinant genomic DNA. Corrected signals for the glutathione *S*-transferases and albumin were normalized relative to the corrected pRGAPDH-13 signal for the respective filter. To enable an indirect comparison of transcription rates of GST and albumin, the corrected and normalized values were divided by the respective gene length giving the relative proportion of transcripts produced per unit from that gene, to the number of GAPDH transcripts produced per unit time.

3. RESULTS

3.1. *Transcription measurements in isolated nuclei*

To determine whether changing steady-state levels of GST 1/2, 3/4 and 7 mRNA in conventional and co-cultured adult rat hepatocytes [16,17] were due to altered stability of the RNAs or a change in the rate of gene transcription, GST transcription was analyzed by a run-on transcription assay. Hepatocytes were maintained for 4 days in conventional culture, and 4 and 12 days in co-culture treated with Nic, DMSO or with or without FCS. Nuclei were isolated and nascent RNA elongated in vitro in the presence of [^{32}P]UTP. Labelled RNA was hybridized to excess DNA from various genes that had been immobilized on nitrocellulose filters. Representative slot blots from these experiments are illustrated in Fig. 1. Results, quantified by densitometry, are presented in Table I. The relative rates of transcription of the GST 1/2, 3/4 and 7 and albumin genes have been calculated relative to transcription of the constitutive gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), after subtracting the background signal.

3.1.1. Conventional culture

Compared to the value for freshly isolated hepatocytes, transcription of the GST 1/2 genes decreases whatever the culture media conditions. In agreement with previously reported increased GST 1/2 and 3/4 steady-state mRNA levels obtained after 4 and 6 days in conventional cultures supplemented with Nic [16,17], hepatocytes cultured under these conditions also maintain the highest transcriptional activity for subunits 1/2, and an approximately 4-fold induction of the GST 3/4 mRNA elongation is detected when compared to freshly isolated hepatocytes. Increased 1/2 and 3/4 transcripts [16,17] in DMSO-treated hepatocyte cultures are not a consequence of increased transcriptional activity.

GST 7 transcription was not detected and was very low in freshly isolated hepatocytes and DMSO-treated cultures, respectively. This contrasts with a markedly enhanced transcription of this gene when cells in vitro were maintained in standard medium or treated with FCS or with Nic. Albumin transcription was not markedly affected by seeding the hepatocytes in conventional culture.

3.1.2. Co-culture

Co-cultured hepatocytes maintained for 4 days retained 60–70% of the initial GST 1/2 transcriptional rate, except when the cultures were deprived of FCS (30%). The GST 1/2 RNA elongation is decreased to a very low or undetectable level after 12 days of co-culture.

Transcription of the GST 3/4 genes is well maintained under all culture conditions up to 12 days. Compared to the value obtained for freshly isolated hepatocytes, a maximal 4-fold induction is observed, occurring when Nic is added to the medium (day 4). Values were only lower than the initial level when cells were treated with and without FCS after 4 and 12 days, respectively.

GST 7 transcription was detected in 4- and 12-day-old co-cultured hepatocytes whatever their treatment, but it was significantly reduced in the presence of DMSO.

Rat liver epithelial cells demonstrate transcriptional activity of the GST 3/4 and 7 genes (Table I). However, the contribution of these cells to the GST μ and π transcriptional activity of the co-cultures is negligible as demonstrated when hepatocytes were separated from the epithelial cells after the respective co-culture period (results not shown).

Albumin gene transcription is enhanced in both 4- and 12-day co-cultured hepatocytes. Rat liver epithelial cells had no detectable albumin gene transcriptional activity (Table I).

4. DISCUSSION

Adult rat hepatocytes, often used to study drug metabolism and detoxification mechanisms in vitro [25], are known to undergo differential changes when seeded in culture [13]. In particular, changes in cytochrome P-450-dependent enzymatic activities and correlated gene expression have been frequently studied during recent years [26–28]. Some previous investigations demonstrate that the glutathione *S*-transferase isoenzyme profile and RNA transcripts of the different subunits also change when hepatocytes are removed from their natural environment and that variations occur depending on the type of culture and media conditions used [15–17,19].

The aim of the present work was to study GST 1/2, 3/4 and 7 gene transcription in conventionally and co-cultured adult rat hepatocytes using medium conditions investigated in previous studies where GST activity, protein levels and steady-state levels of mRNA were reported [15–17]. Transcription of the albumin gene was also assessed in isolated nuclei at different stages to serve as a reference. Albumin transcription was not detected in rat liver epithelial cells. The relative rate of transcription of this gene was, in agreement with the literature [30], not changed or enhanced once the hepatocytes were seeded in culture and co-culture, respectively.

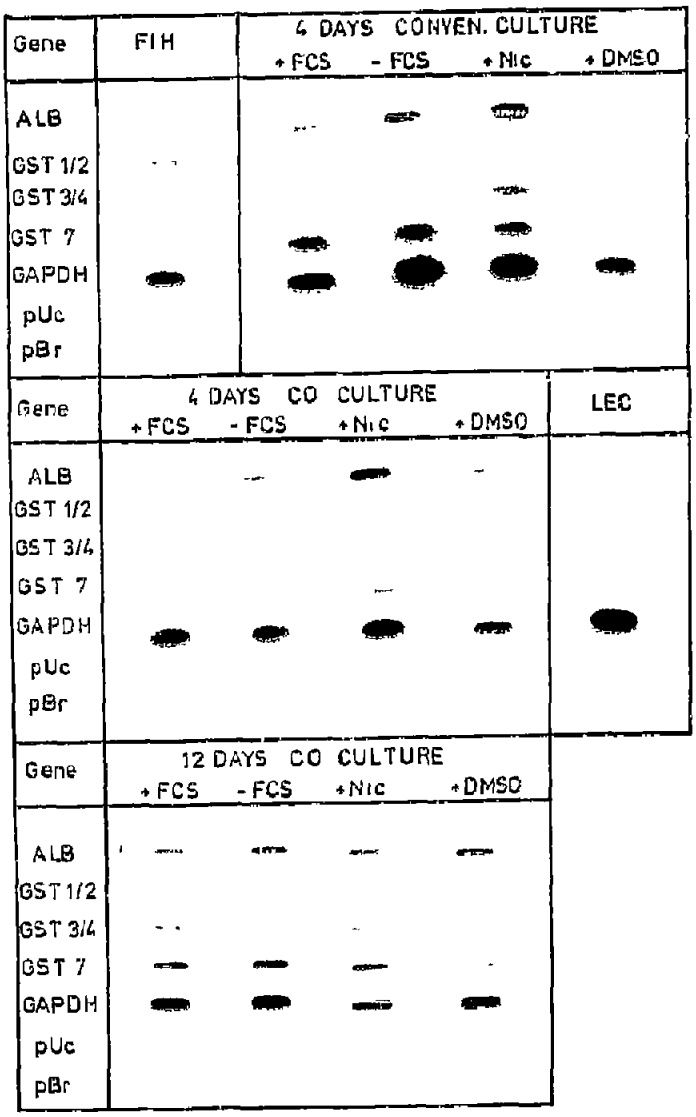

Fig. 1. Transcriptional activity of conventionally- and co-cultured adult rat hepatocytes. Transcriptional analysis in nuclei isolated from freshly isolated hepatocytes (FIH), 4-days conventionally cultured hepatocytes, 4- and 12-days co-cultured hepatocytes and rat liver epithelial cells (LEC). Hepatocytes were maintained in culture with (+FCS) and without fetal calf serum (−FCS) or with nicotinamide (Nic) or dimethylsulphoxide (DMSO) as described in section 2. Nuclei were isolated, and nascent RNA chains were elongated in vitro and hybridized to immobilized DNA from the following genes: albumin (ALB), glutathione *S*-transferase subunits 1 and 2 (GST 1/2), glutathione *S*-transferase subunits 3 and 4 (GST 3/4), glutathione *S*-transferase subunit 7 (GST 7), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pUC and pBR.

Subunit 7, barely detectable in normal adult rat liver and not found in freshly isolated hepatocytes, is expressed when hepatocytes are seeded in culture [15,31]. Results from this study demonstrate that the regulation of expression in vitro of this particular subunit occurs at the transcriptional level. Indeed, in the presence of

DMSO, an extremely low transcriptional activity is observed resulting in a barely detectable level of protein when staining the DMSO-treated hepatocytes for this subunit (results not shown). This agrees with previous studies reporting that the de novo synthesis of subunit 7 [15] and its corresponding steady-state mRNA level were markedly decreased when the parenchymal cells in culture were treated with 2% of this organic compound.

The expression of subunit 7 is also detected in fetal liver parenchymal cells [10] and at early stages of hepatocarcinogenesis [11]. The mechanism of down-regulation of GST 7 which occurs after birth and its re-expression during liver transformation is unknown at present. It is also unclear whether the re-expression of this subunit in cultured hepatocytes and in preneoplasia is regulated by the same mechanism. The finding that in culture [32], as well as in preneoplastic nodules [33] the appearance of GST 7 is correlated with expression of oncogenes, suggests this may be an underlying factor. Secondly, the question arises as to whether some relation exists between the de novo expression of subunit 7 and its high peroxidase activity [34], especially since it has been found that DMSO, being a free radical scavenger, inhibits its expression. Sato [35] suggested that the expression of GST π may be related to the prevention of lipid peroxidation since this latter process has been considered to play an important role during tumor promotion. Also, an increased production of lipid hydroperoxides has been found in cultured hepatocytes [36].

Decreasing GST 1/2 [16] and increasing GST 3/4 [17] transcripts are the result of varying transcriptional activities of the respective genes in conventionally- and co-cultured rat hepatocytes. These observations indicate that the expression of these GST families in cultured hepatocytes is controlled primarily at the level of transcription. However, this is not the case when DMSO is added to the cells. In its presence, observed especially in conventional culture, the transcriptional activity of the GST 1/2 and 3/4 genes decreases compared to the value obtained for freshly isolated hepatocytes, while the respective steady-state mRNA levels were substantially increased [16,17]. This probably indicates that DMSO treatment, in contrast to the other agents studied, stabilizes the GST mRNAs in vitro.

Compared to conventional cultures, 4-day co-cultured hepatocytes maintain α class gene transcription at a higher level, whatever the medium conditions. Nevertheless, a drastic decrease in this particular transcriptional activity is observed after 12 days of co-culture. At the same time, stage μ class transcription still occurs at an elevated level compared to the initial level. Mechanisms by which α and μ class gene transcription are dramatically lowered or increased, respectively, in cultured hepatocytes are not clear. As suggested by Listow-

Table I

GST and albumin transcription in isolated nuclei prepared from conventionally and co-cultured adult rat hepatocytes

	Vector signal		Hybridization signal* (corrected towards vector)					Relative rate of transcription**			
	pUC	pBR	GST1/2	GST3/4	GST7	Alb	GAPDH	GST1/2	GST3/4	GST7	Alb
FIH	0.23	0.25	0.20	0.08	ND	0.20	1.16	0.44	0 21	ND	0 03
LEC	0.24	0.31	ND	0.12	0.30	0.02	2.62	ND	0.14	0.16	0.00
4-Days conventional culture											
+FCS	0.42	0.43	0 05	0.07	1 05	0.48	1.78	0.07	0.12	0.83	0.05
−FCS	0 38	0.43	0.04	0 12	1.22	0.59	3 14	0.03	0.12	0 55	0.04
+NIC	0.12	0.15	0.12	0.78	0.77	0.81	2.67	0 12	0.93	0.41	0.06
+DMSO	0.27	0 24	0.04	0.06	0.02	0.21	1.32	0.07	0.14	0.02	0.03
4-Days co-culture											
+FCS	0 10	0 04	0 15	0.07	0 18	0.31	1.49	0.25	0.15	0.17	0.04
−FCS	0 29	0.36	0.06	0 08	0.26	0 63	1.04	0.14	0.24	0 36	0.11
+NIC	0.16	0.14	0.20	0.49	0.39	1.17	1.86	0.28	0 83	0.30	0 11
+DMSO	0.15	0.15	0.11	0.12	0.05	0.39	0.92	0.30	0.41	0.08	0.08
12-Days co-culture											
+FCS	0.67	0 90	0.14	0 35	1 17	0.72	3.29	0.11	0.34	0.50	0.04
−FCS	1.14	1.42	ND	0.10	1.11	0 44	3.83	ND	0.08	0.41	0.02
+NIC	0.62	1.19	ND	0.20	1.74	0.56	2.30	ND	0.28	1.07	0.05
+DMSO	0.60	1.02	0 05	0.37	0.55	1.21	3.14	0.04	0.38	0.25	0.07

All numerical values refer to optical density units. FIH and LEC refer to values obtained from freshly isolated hepatocytes and rat liver epithelial cells, respectively. Alb, albumin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase, NIC, nicotinamide, FCS, fetal calf serum, DMSO, dimethylsulphoxide; ND, signal not detectable.

*Data has been corrected for non-specific hybridization by subtraction of vector signal.

**Glutathione *S*-transferase and albumin densitometric signals were corrected for the fraction of primary gene transcripts hybridizable to the genomic DNA, normalized relative to GAPDH, and converted to relative rates of transcription as described in section 2.

sky et al. [37], the decrease in GST 1 transcripts and transcriptional activity may reflect lack of humoral factors, as well as components in the medium that ordinarily induce subunit 1 in hepatocytes. Indeed, they recently demonstrated that levels of liver GST 1 transcripts in adrenalectomized rats were restored by administration of glucocorticoids [37]. It was also observed by our group that the GST 1/2 transcriptional activity [38] and corresponding steady-state mRNA levels [29] were induced in vitro by treating the hepatocytes with various xenobiotics, e.g. phenobarbital.

In conclusion, this is the first study to demonstrate that the regulation of expression of GST proteins in cultured hepatocytes occurs at different levels, depending on the media and culture conditions. Indeed, evaluating the discrepancy between transcriptional activity of GST α and μ class genes and the corresponding steady-state mRNA levels of the DMSO-treated hepatocytes, demonstrates that this organic compound effects expression of the main GST subunits (subunits 1–4) at the post-transcriptional level. This observation contrasts with the expression of GST subunits in hepatocytes cultured with or without FCS and nicotinamide, where regulation of expression occurs at the transcriptional level.

Acknowledgements: We would like to thank Prof. B. Ketterer and Dr J. Taylor for the generous gift of the GST cDNA probes, pGSTr 155, pGSTr7 and JT9L. V.Y.'s stay at the University of Western Australia was supported by the International Union Against Cancer (Yamagiwa–Yoshida Memorial International Cancer Study Grant) and NFWO (Fund for Medical Scientific Research). We thank Seneca Veerle for technical assistance.

REFERENCES

[1] Chasseaud, L.F. (1979) Adv. Cancer Res. 29, 175–274.
[2] Kamisaka, K., Listowsky, I., Gatmaitan, Z. and Arias, I.M. (1975) Biochemistry 14, 2175–2180.
[3] Ketterer, B. (1986) Xenobiotica 16, 957–973.
[4] Tsuchida, S., Izumi, T., Shimizu, T., Ishikawa, T., Hatayama, I., Satoh, K. and Sato, K. (1987) Eur. J. Biochem. 170, 159–164.
[5] Benson, A.M., Talalay, P., Keen, J.H. and Jakoby, W.B. (1977) Proc. Natl. Acad. Sci. USA 74, 158–162.
[6] Mannervik, B. and Danielson, U.H. (1988) CRC Crit. Rev. Biochem. 23, 283–337.
[7] Mannervik, B., Alin, P., Guthenberg, C., Jensson, H., Tahir, M.K., Warholm, M. and Joernvallh, H. (1985) Proc. Natl. Acad. Sci. USA 82, 7202–7206.
[8] Meyer, D.J., Coles, B., Pemble, S., Gilmore, K.S., Fraser, G.M. and Ketterer, B. (1991) Biochem. J. 274, 409–414.
[9] Ketterer, B. (1988) Mut. Res. 202, 343–361.
[10] Abramovitz, M. and Listowsky, I. (1988) Xenobiotica 18, 1249–1254.
[11] Satoh, K., Kitahara, A., Soma, Y., Inaba, Y., Hatayama, I. and Sato, K. (1985) Proc. Natl. Acad. Sci. USA 82, 3964–3968.
[12] Vandenberghe, Y., Glaise, D., Meyer, D.J., Guillouzo, A. and Ketterer, B. (1988) Biochem. Pharmacol. 37, 2482–2485.
[13] Guguen-Guillouzo, C. and Guillouzo, A. (1983) Mol. Cell Biochem. 53/54, 35–56.
[14] Vandenberghe, Y., Ratanasavanh, D., Glaise, D. and Guillouzo, A. (1988) In Vitro Cell. Dev. Biol. 24, 281–288.
[15] Vandenberghe, Y., Foriers, A., Rogiers, V. and Vereruysse, A. (1990) Biochem. Pharmacol. 39, 685–690.
[16] Vandenberghe, Y., Morel, F., Pemble, S., Taylor, J.B., Rogiers, V., Ratanasavanh, D., Vereruysse, A., Ketterer, B. and Guillouzo, A. (1990) Mol. Pharmacol. 37, 372–376.
[17] Morel, F., Vandenberghe, Y., Pemble, S., Taylor, J.B., Ratanasavanh, D., Ketterer, B. and Guillouzo, A. (1989) FEBS Lett. 258, 99–102.
[18] Guguen, C., Guillouzo, A., Boisnard, M., Le Cam, A. and Bourel, M. (1975) Biol. Gastroenterol. 8, 223–231.
[19] Becker, P., Renkawitz, R. and Schütz, G. (1984) EMBO J. 3, 2015–2020.
[20] Shelly, L.L., Tynan, W., Schmid, W., Schuetz, G. and Yeoh, G.C. (1989) J. Cell Biol. 109, 3403–3410.
[21] Sargent, T.D., Jagodzinski, L.L., Yang, M. and Bonner, J. (1981) Mol. Cell Biol. 1, 871–883.
[22] Taylor, J.B., Craig, R.K., Beale, D. and Ketterer, B. (1984) Biochem. J. 219, 223–231.
[23] Pemble, S., Taylor, J.B. and Ketterer, B. (1986) Biochem. J. 240, 885–889.
[24] Fort, P., Marty, L., Piechaczyk, S., el Sabrouty, S., Dani, C., Jeanteur, P. and Blanchard, J.M. (1985) Nucleic Acids Res. 13, 1431–1442.
[25] Guillouzo, A. (1986) in: Isolated and Cultured Hepatocytes (Guillouzo, A. and Guguen-Guillouzo, C. eds.) pp. 311–331, John Libbey Eurotext Ltd., London.
[26] Silver, G., Reid, L.M. and Krauter, K.S. (1990) J. Biol. Chem. 265, 3134–3138.
[27] Dickens, M. and Peterson, R.E. (1980) Biochem. Pharmacol. 29, 1231–1238.
[28] Newman, S. and Guzelian, P.S. (1982) Proc. Natl. Acad. Sci. USA 79, 2922–2926.
[29] Vandenberghe, Y., Morel, F., Foriers, A., Ketterer, B., Vereruysse, A., Guillouzo, A. and Rogiers, V. (1989) FEBS Lett. 251, 59–64.
[30] Fraslin, J.M., Kneip, B., Vaulont, S., Glaise, D., Munnich, A. and Guguen-Guillouzo, C. (1985) EMBO J. 4, 2487–2491.
[31] Abramovitz, M., Ishigaki, S. and Listowsky, I. (1989) Hepatology 9, 235–239.
[32] Guguen-Guillouzo, C., Baffet, G., Etienne, P.L., Glaise, D., Defer, N., Corral, M., Coreos, D. and Kruh, J. (1988) in: Experimental Hepatocarcinogenesis (Roberfroid, M.B. and Préat, V. eds.) pp. 245–253, Plenum Press, New York.
[33] Sato, K., Satoh, K., Tsuchida, S., Hatayama, I., Tamai, K. and Shen, H. (1990) in: Glutathione S-Transferases and Drug Resistance (Hayes, J.D., Pickett, C.B. and Mantle, T.J. eds.) pp. 389–398, Taylor and Francis, London.
[34] Meyer, D.J., Beale, D., Hong Tan, K.H., Coles, B. and Ketterer, B. (1985) FEBS Lett. 184, 139–143.
[35] Sato, K. (1989) Adv. Cancer Res. 52, 205–255.
[36] Kalopissis, A.D. and Griglio, S. (1986) in: Isolated and Cultured Hepatocytes (Guillouzo, A. and Guguen-Guillouzo, C. eds.) pp. 113–153, John Libbey Eurotext Ltd., London.
[37] Listowsky, I., Campbell, E., Takahashi, Y., Ishigaki, S., Abramovitz, M. and Homma, H. (1990) in: Glutathione S-Transferases and Drug Resistance (Hayes, J.D., Pickett, C.B. and Mantle, T.J. eds.) pp. 272–279, Taylor and Francis, London.
[38] Vandenberghe, Y., Tee, L., Morel, F., Rogiers, V., Guillouzo, A. and Yeoh, G. (1991) FEBS Lett. 284, 103–108.