Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5091–5095, August 1984
Biochemistry

Regulation of glutathione transferase and DT-diaphorase mRNAs in persistent hepatocyte nodules during chemical hepatocarcinogenesis

(immunoprecipitation/RNA gel blot hybridization/neoplastic transformation)

CECIL B. PICKETT*, JACINTA B. WILLIAMS*, ANTHONY Y. H. LU†, AND ROSS G. CAMERON‡

*Department of Biochemical Regulation and †Animal Drug Metabolism, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065; and ‡Department of Pathology, University of Toronto, Toronto, Canada M5S 1A8

Communicated by Roy Vagelos, May 4, 1984

ABSTRACT **We have utilized cDNA probes and *in vitro* translation analysis to quantitate the levels of rat liver glutathione transferase (glutathione *S*-aralkyltransferase; RX:glutathione R-transferase, EC 2.5.1.18) and DT-diaphorase [NAD(P)H:quinone-acceptor oxidoreductase, EC 1.6.99.2] mRNAs in persistent hepatocyte nodules induced by chemical carcinogens. Our results indicate that within the nodules, glutathione transferase mRNAs specific for the Ya/Yc and Yb subunits are increased 3-fold and 5-fold, respectively, over the levels observed in normal liver or in the liver tissue surrounding the nodules. Similarly, the level of DT-diaphorase mRNA is increased 5- to 7-fold within the nodules as compared to surrounding liver tissue or normal liver. When animals were administered 3-methylcholanthrene, a typical inducer of these mRNAs in normal animals, a further increase in the glutathione transferase Yb mRNA(s) and DT-diaphorase mRNA was observed in the nodules; however, the Ya/Yc mRNA levels remained unaffected. Our data indicate that during chemically induced neoplastic transformation, the mRNA levels for the Yb subunit of glutathione transferase and DT-diaphorase are increased in the nodules but still retain the capacity to be regulated by 3-methylcholanthrene. Although the glutathione transferase Ya/Yc mRNAs are also increased in the nodules, they lost their ability to be regulated by 3-methylcholanthrene. These latter data suggest that within the nodules there is a specific defect in the regulatory mechanism(s) that leads to an induction of the Ya/Yc mRNAs in normal tissue by xenobiotics.**

The administration of a single initiating dose of diethylnitrosamine to rats followed by a low level of 2-acetylaminofluorene plus a partial hepatectomy induces foci of altered hepatocytes, which have been referred to as persistent hepatocyte nodules, hyperplastic nodules, or preneoplastic nodules (1). Persistent hepatocyte nodules are thought to represent precursors for the subsequent development of hepatocellular carcinomas and, thus, have been utilized as a model system to study chemically induced neoplastic transformation (1, 2). Biochemically the nodules have elevated levels of various drug-metabolizing enzymes {e.g., glutathione transferases (glutathione *S*-aralkyltransferase; RX:glutathione R-transferase, EC 2.5.1.18), DT-diaphorase [NAD(P)H:quinone-acceptor oxidoreductase, EC 1.6.99.2], and epoxide hydrolase}; however, cytochrome P-450 levels appear to be depressed (3–11). The elevated levels of some phase II drug-metabolizing enzyme activities and a decreased level of cytochrome P-450 are consistent with the observation that persistent hepatocyte nodules are more resistant to the cytotoxic effects of carcinogens as compared to normal liver (12). In fact, Farber (2) has hypothesized that an early or even first biological cellular event in liver carcinogenesis is the induction in a rare hepatocyte of resistance to the cytotoxic effect of carcinogens.

In order to understand the mechanisms of chemical carcinogenesis, a basic understanding of the regulation of the enzymes involved in carcinogen metabolism is essential. In the present study, we have focused on defining the molecular events that are responsible for the increase of glutathione transferase and DT-diaphorase enzyme activities in nodular tissue. We utilized cDNA probes complementary to the mRNAs of the rat liver glutathione transferases as well as *in vitro* translation and immunoprecipitation (in the case of DT-diaphorase) to measure mRNA levels in nodular tissue, surrounding liver tissue, and normal tissue. We also have examined whether glutathione transferase and DT-diaphorase mRNAs can be regulated in nodular tissue by 3-methylcholanthrene (3MC) a typical inducer of these mRNAs in normal animals. Our results demonstrate that glutathione transferase and DT-diaphorase mRNAs are increased in the nodules relative to the liver tissue surrounding the nodules. Furthermore, it appears that the level of glutathione transferase Yb mRNA(s) and the DT-diaphorase mRNA in nodular tissue can be augmented by 3MC administration, whereas the Ya/Yc mRNA levels remain unaffected.

METHODS

Persistent Hepatocyte Nodule Induction. Male Sprague–Dawley rats from Charles River Breeding Laboratories were utilized in all experiments. Persistent hepatocyte nodules were induced by using the Solt–Farber model (1). Diethylnitrosamine was given at a dose of 200 mg/kg of body weight i.p. in 0.9% NaCl. After a 1- to 2-wk period, animals were given three doses at daily intervals of 2-acetylaminofluorene (20 mg/kg of body weight, intragastrically), followed on day 4 by a partial hepatectomy. In order to induce large nodules, the selection procedure was repeated two or three times using three doses of 2-acetylaminofluorene at daily intervals, followed by carbon tetrachloride, which served as a stimulus for cell proliferation (13). Carbon tetrachloride was dissolved 1:1 in corn oil and was administered intragastrically at a dose of 0.4 ml/100 g of body weight. In the induction studies, rats received 3MC (40 mg/kg of body weight) for 5 days and were sacrificed 24 hr after the final injection.

RNA Isolations, Cell-Free Protein Synthesis, and Immunoprecipitation of DT-Diaphorase and Cytochrome P-448. Total rat liver RNA was isolated from nodular tissue or the tissue surrounding the nodules by the guanidine thiocyanate method of Chirgwin *et al.* (14). Poly(A)$^+$ RNA was isolated from the total rat liver RNA preparations by oligo(dT)-cellulose chromatography as described (15). Total liver poly(A)$^+$ RNA was translated for 90 min at 30°C in a micrococcal nuclease-treated rabbit reticulocyte lysate system. The reaction mixture has been described in detail (15).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: 3MC, 3-methylcholanthrene.

DT-diaphorase and cytochrome P-448 were recovered from the translation mixtures by indirect immunoprecipitation utilizing protein A-sepharose. The purification of DT-diaphorase (16) and cytochrome P-448 (17), and the preparation of antibodies against the purified proteins have been described (18). For the immunoprecipitation of DT-diaphorase, translation mixtures were diluted 1:5 with 190 mM NaCl/50 mM Tris·HCl, pH 7.5/6 mM EDTA/1.0% Triton X-100/1.0% sodium deoxycholate and centrifuged at 100,000 × *g* for 1 hr to pellet ribosomes. Ten microliters of rabbit-anti DT-diaphorase IgG were added to the supernatant, followed by 100 μl of protein A-sepharose. The mixture was incubated overnight at 4°C with end-to-end rotation. The immune complex/protein A-sepharose mixture was pelleted by centrifugation and washed five times with phosphate-buffered saline containing 0.1% $NaDodSO_4$ and 0.5% Nonidet P-40. The antigen was eluted from the antibody by boiling in $NaDodSO_4$/polyacrylamide sample buffer containing 10 mM dithiothreitol. The protein A-sepharose was removed by centrifugation, and the supernatant containing the antigen was subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis.

The radiolabeled DT-diaphorase was identified by fluorography utilizing ENLIGHTNING (New England Nuclear). Cytochrome P-448 was immunoprecipitated from *in vitro* translation mixtures as described (19). Briefly, translation mixtures were made 2% in $NaDodSO_4$, boiled for 3 min, and diluted with 4 volumes of 2.5% Triton X-100/190 mM NaCl/50 mM Tris·HCl, pH 7.4/6 mM EDTA. The mixture was preincubated for 2 hr with 10 μl of IgG obtained prior to immunization (preimmune IgG) and 100 μl of protein A-sepharose to decrease nonspecific binding. The preimmune IgG–protein A-sepharose complex was removed by centrifugation, and 10 μl of rabbit anti-rat cytochrome P-448 and 100 μl of protein A-sepharose were added to the supernatant. The immune complexes bound to the protein A-sepharose were recovered by centrifugation and elution as described above for DT-diaphorase. In order to quantitate the level of translatable DT-diaphorase, the regions of the gels containing the radiolabeled protein were excised and dissolved in 60% perchloric acid/30% hydrogen peroxide at 60°C. Aquasol-2 was added to the solubilized gel slices, and total radioactivity was determined by liquid scintillation counting. Background radioactivity was determined by excising gel regions above and below the radiolabeled DT-diaphorase and solubilizing the slices as described above. The values obtained were then subtracted from the radioactivity obtained in the slices containing DT-diaphorase.

Analysis by RNA Gel Blot Hybridization. For the RNA gel blot hybridizations, two cDNA clones complementary to the rat liver glutathione transferases were utilized. The cDNA clones, pGTB38 and pGTA/C36, have been characterized by hybrid-selected translation and restriction endonuclease mapping (20). Clone pGTB38 harbors a 950-base-pair cDNA insert that by hybridization selects the Ya and Yc mRNAs of the rat liver glutathione transferase, whereas pGTB/C36 harbors a 400-base-pair cDNA insert that by hybridization selects the Yb mRNA(s). The sequence of the Ya/Yc clone, pGTB38, has been determined, and the deduced amino acid sequence of the corresponding protein that represents a Ya subunit has been determined (20). Total rat liver poly(A)$^+$ RNA (5 μg) was electrophoresed in 1.5% agarose gels containing 10 mM methylmercury(II) hydroxide (21) and transferred to diazobenzyloxymethyl paper as described by Alwine *et al.* (22). After transfer, filters were treated prior to hybridization overnight at 42°C in 50% formamide/25 mM sodium phosphate, pH 6.5/0.75 M NaCl/75 mM trisodium citrate/0.2% $NaDodSO_4$/0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone/1% glycine/1 mg of sonicated denatured salmon sperm DNA per ml. After the prehybridization treatment, the solution was removed and replaced with a fresh aliquot of the same solution containing 4 × 10^6 dpm of ^{32}P-labeled pGTB38 or pGTA/C36 and incubated overnight at 42°C. After incubation, the filters were washed twice in 0.36 M NaCl/20 mM sodium phosphate, pH 7.0/2 mM EDTA/0.1% $NaDodSO_4$ at room temperature and twice with 18 mM NaCl/1 mM sodium phosphate, pH 7.0/0.1 mM EDTA/0.1% $NaDodSO_4$ for 30 min at 50°C. The diazobenzyloxymethyl paper was allowed to dry and then exposed to Kodak AR2 x-ray film. The audioradiograph was scanned with a densitometer to quantitate relative mRNA levels.

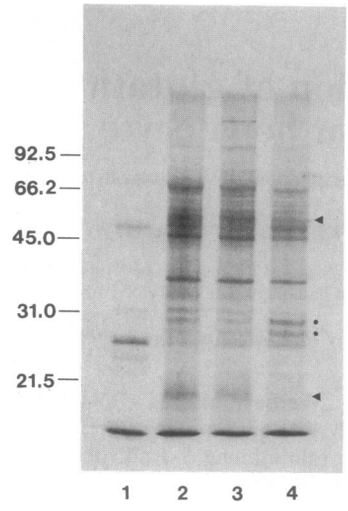

FIG. 1. [^{35}S]Methionine-labeled translation products directed by poly(A)$^+$ RNA isolated from normal rat liver (lane 2), liver tissue surrounding persistent hepatocyte nodules (lane 3), and nodular tissue (lane 4). Lane 1 represents endogenous protein synthesis in the absence of added mRNA. The arrowheads indicate polypeptide(s) whose levels are decreased in the translations programmed with mRNA isolated from persistent hepatocyte nodules, whereas the dots represent those polypeptides whose levels are increased. The molecular weight markers (shown ×10^{-3}) are phosphorylase B (M_r 92,000), bovine serum albumin (M_r 66,200), ovalbumin (M_r 45,000), carbonic anhydrase (M_r 30,000), and soybean trypsin inhibitor (M_r 21,500).

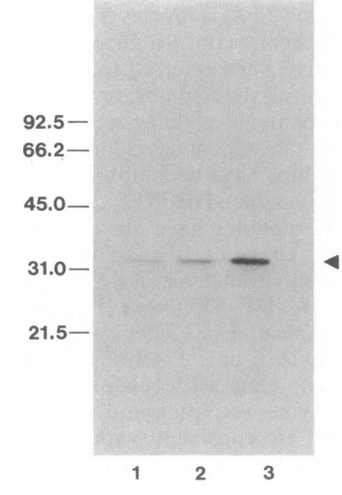

FIG. 2. Fluorograms of $NaDodSO_4$/polyacrylamide gel electrophoresis of DT-diaphorase immunoprecipitated from translation mixtures programmed with poly(A)$^+$ RNA isolated from normal liver (lane 1), surrounding liver tissue (lane 2), and nodular tissue (lane 3). The arrow shows the migration of purified DT-diaphorase in the $NaDodSO_4$/polyacrylamide gel. The molecular weight markers (shown ×10^{-3}) are identical to those presented in Fig. 1.

Table 1. DT-diaphorase mRNA levels in normal liver, surrounding liver tissue, and nodular tissue isolated from untreated rats

Tissue	Total radioactivity in immunoprecipitated DT-diaphorase, cpm	
	Exp. 1	Exp. 2
Normal liver	1161 (1.0)	788 (1.0)
Surrounding liver	1825 (1.6)	1476 (1.9)
Nodules	6153 (5.3)	5769 (7.3)

For experiment 1, the total input cpm used in the immunoprecipitation reaction was 28×10^6, whereas in experiment 2 the total input cpm was 31×10^6. The values in parentheses represent the fold increase over normal liver tissue. Liver tissue from three to five animals was pooled for each experiment.

***In Vitro* Labeling of DNA.** Cloned cDNA sequences were labeled *in vitro* with [^{32}P]dCTP (≈800 Ci/mmol; 1 Ci = 37 GBq) by nick-translation (23).

RESULTS

Quantitation of mRNA Levels for DT-Diaphorase and Glutathione Transferases in Normal, Nodular, and Surrounding Liver Tissue. In order to quantitate the level of mRNA for DT-diaphorase and the glutathione transferases, total rat liver RNA was isolated from nodular tissue, surrounding liver tissue, and normal tissue by the guanidine thiocyanate procedure of Chirgwin *et al.* (14). The total RNA was fractionated by oligo(dT)-cellulose chromatography and either translated *in vitro* by using the rabbit reticulocyte cell-free translation system or subjected to agarose gel-electrophoresis in the presence of methylmercury(II) hydroxide. Based on both of these assays, the mRNA from all tissues was judged to be intact. Autoradiograms of the total translation products are illustrated in Fig. 1. As can be seen from this figure, polypeptides with molecular weights ranging from 10,000 to >100,000 were synthesized in the lysates programmed with the various RNA preparations. Of particular interest were the differences that could be observed grossly between the translations programmed with the mRNA isolated from the nodules (Fig. 1, lane 4) as compared to the corresponding

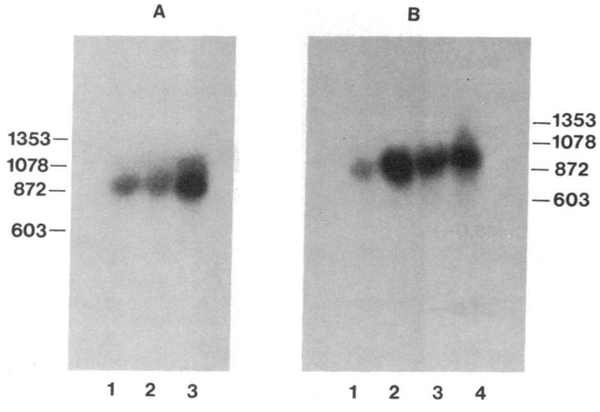

FIG. 3. (*A*) RNA gel blot hybridization of the glutathione transferase Ya/Yc cDNA clone, pGTB38, to liver mRNA (5 μg) isolated from normal rat liver (lane 1), surrounding liver tissue (lane 2), and nodular tissue (lane 3). The molecular weight markers represent restriction fragments from a *Hae* III digest of θX174RF DNA. (*B*) RNA gel blot hybridization of glutathione transferase Ya/Yc cDNA clone, pGTB38, to liver mRNA isolated from untreated rats (lane 1), liver mRNA isolated from 3MC-treated rats (lane 2), surrounding liver tissue mRNA isolated from 3MC-treated rats (lane 3), and nodular tissue mRNA isolated from 3MC-treated rats (lane 4). The molecular weight markers are identical to those in *A*.

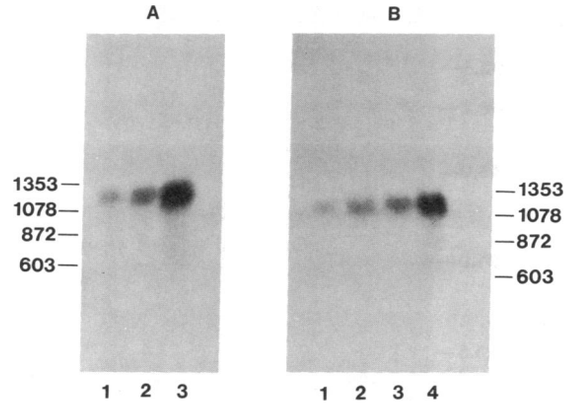

FIG. 4. (*A*) RNA gel blot hybridization of the glutathione transferase Yb cDNA clone, pGTA/C36, to mRNA (5 μg) isolated from normal rat liver (lane 1), surrounding liver tissue (lane 2), and nodular tissue (lane 3). The molecular weight markers are identical to those presented in Fig. 3*A*. (*B*) RNA gel blot hybridization of glutathione transferase Yb cDNA clone, pGTA/C36, to liver mRNA isolated from untreated rats (lane 1), liver mRNA isolated from 3MC-treated rats (lane 2), surrounding liver tissue mRNA isolated from 3MC-treated rats (lane 3), and nodular tissue mRNA isolated from 3MC-treated rats (lane 4). The molecular weight markers are identical to those presented in Fig. 3*A*.

mRNA isolated from normal liver tissue (Fig. 1, lane 2) or surrounding liver tissue (Fig. 1, lane 3). The arrowheads point to polypeptides of M_rs 19,000 and 52,000 that were diminished in the translations programmed with nodule mRNA, whereas the closed circles represent polypeptides whose syntheses were elevated.

To assess the level of specific mRNAs (i.e., DT-diaphorase and glutathione transferase mRNAs) in the nodules, we used both *in vitro* translation analysis and specific immunoprecipitation as well as RNA blot hybridization. Utilizing the *in vitro* translation assay, we found that the level of functional DT-diaphorase mRNA was increased 5- to 7-fold in persistent hepatocyte nodules, whereas the mRNA level in surrounding liver tissue was only increased slightly (≈1.8-fold) as compared to normal liver tissue (Fig. 2 and Table 1). The slight increase in mRNA level in the surrounding liver may be due to a few microscopic nodules in this tissue. For the RNA blot-hybridization experiments, we utilized two cDNA clones, pGTB38 and pGTA/C36, which selectively hybridize with the Ya/Yc mRNAs and Yb mRNA of the rat liver glutathione transferases, respectively (20). Utilizing these cDNA clones, we found that the level of the Ya/Yc mRNAs and the Yb mRNA(s) of the rat liver glutathione transferases were increased 2.8-fold and 5.3-fold, respectively, in the nodules, with only a slight increase being observed in the surrounding liver tissue (Figs. 3*A* and 4*A* and Table 2).

Induction of Glutathione Transferase and DT-Diaphorase mRNAs in Persistent Hepatocyte Nodules by 3MC. Since DT-

Table 2. Glutathione transferase mRNA levels in normal liver tissue, surrounding liver tissue, and nodular tissue isolated from untreated rats

Tissue	Relative mRNA level	
	Ya/Yc mRNA	Yb mRNA
Normal liver	1.0	1.0
Surrounding liver	1.2	1.8
Nodules	2.8	5.3

The relative levels of mRNA specific for each subunit was determined by scanning the x-ray films (Figs. 3*A* and 4*A*) obtained from the RNA gel blot hybridizations with a densitometer in order to determine the area represented by each hybridization signal. The mRNA level in normal liver was arbitrarily assigned a value of 1.

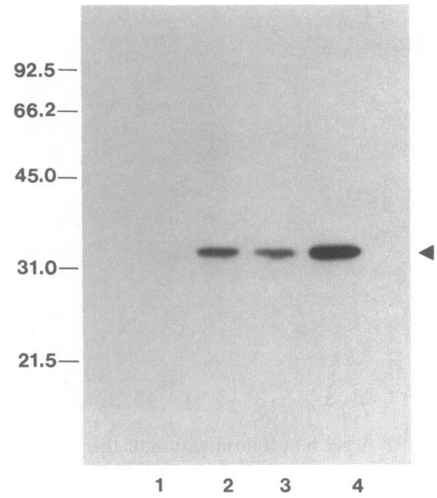

FIG. 5. Fluorogram of $NaDodSO_4$/polyacrylamide gel electrophoresis of DT-diaphorase immunoprecipitated from translation systems programmed with poly(A)$^+$ RNA isolated from normal rat liver (lane 1), liver isolated from 3MC-treated rats (lane 2), surrounding liver tissue from 3MC-treated rats (lane 3), and nodular tissue from 3MC-treated rats (lane 4). The arrow represents the migration of purified DT-diaphorase on the $NaDodSO_4$/10% polyacrylamide gel. The molecular weight markers (shown $\times 10^{-3}$) are identical to those presented in Fig. 1.

diaphorase and glutathione transferase mRNA levels were increased in the nodules, we asked whether these levels were still regulated by 3MC. We had demonstrated previously that 3MC leads to an increase in both DT-diaphorase mRNA (24) and the Ya/Yc and Yb mRNAs of the glutathione transferase when normal rats are injected with the polycyclic aromatic hydrocarbon (20). After 3MC administration to rats, the level of functional DT-diaphorase mRNA in the nodular tissue was increased 14- to 21-fold over the level in untreated rats (Fig. 5) and was ≈3-fold higher than the level found in either the surrounding liver tissue or in normal animals after treatment with 3MC (Table 3). In RNA blot-hybridization analysis, the Yb mRNA(s) of the rat liver glutathione transferases followed a pattern similar to DT-diaphorase mRNA (Fig. 4*B*). From the RNA blot-hybridization data, the Yb mRNA(s) level in the nodules was increased 13-fold over normal levels after 3MC administration and ≈3.5-fold over the level observed in the surrounding liver tissue of the same animals or in normal animals given the same dose of 3MC (Table 4). In contrast, however, the mRNA level for the glutathione transferase Ya/Yc mRNAs was not increased further in the nodular tissue by 3MC (Fig. 3*B* and Table 4). There was very little difference between the level

Table 3. DT-diaphorase mRNA levels in normal liver tissue, surrounding liver tissue, and nodular tissue isolated from 3MC-treated rats

Treatment	Tissue	Total radioactivity in immunoprecipitated DT-diaphorase, cpm	
		Exp. 1	Exp. 2
None	Untreated liver	742 (1.0)	552 (1.0)
3MC	Normal liver	4,569 (6.2)	2,907 (5.3)
3MC plus nodule induction	Surrounding liver	4,292 (5.8)	3,358 (6.1)
3MC plus nodule induction	Nodules	10,002 (13.5)	11,801 (21.4)

The input cpm used in each immunoprecipitation reaction was 14 × 10^6. The values in parenthesis represent the fold elevation over the level of mRNA in untreated rats.

Table 4. Glutathione transferase mRNA levels in normal liver tissue, surrounding liver tissue, and nodular tissue isolated from 3MC-treated rats

Treatment	Tissue	Relative mRNA level*	
		Ya/Yc mRNA	Yb mRNA
None	Untreated liver	1.0	1.0
3MC	Normal liver	4.3	3.0
3MC plus nodule induction	Surrounding liver	2.9	4.6
3MC plus nodule induction	Nodules	3.2	13.0

*The relative mRNA levels were determined as described in the legend of Table 2.

of the Ya/Yc mRNAs in nodular tissue, surrounding liver tissue, or normal tissue removed from 3MC-treated rats.

Induction of Cytochrome P-448 mRNA in Nodular Tissue. Since 3MC administration led to a dramatic increase in the level of cytochrome P-448 mRNA (19, 25–28), we determined whether this mRNA could be induced in nodular tissue. It should be pointed out that cytochrome P-448 mRNA levels in the nodular tissue, surrounding liver tissue, and normal tissue cannot be detected by the translational assay used in this study (data not shown). Upon treatment of rats with 3MC, a tremendous increase of cytochrome P-448 mRNA occurred in all tissues (Fig. 6). The amount of cytochrome P-448 mRNA in all three tissues was very similar. Therefore, there was no impairment in the ability of 3MC to induce cytochrome P-448 in the nodules.

DISCUSSION

By utilizing the Solt–Farber liver carcinogenesis model (1), persistent hepatocyte nodules can be easily induced, identified, and removed from surrounding liver tissue. These properties make the Solt–Farber model an attractive system

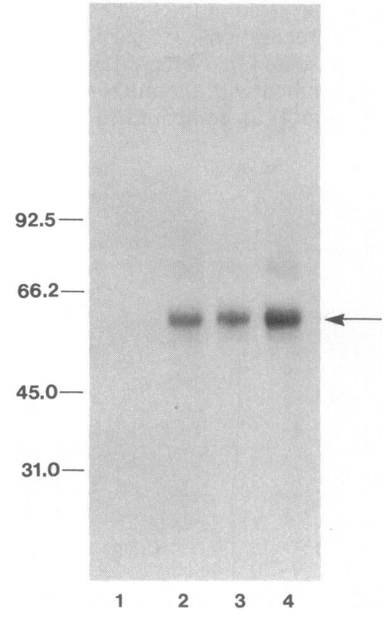

FIG. 6. Fluorogram of $NaDodSO_4$/polyacrylamide gel electrophoresis of cytochrome P-448 immunoprecipitated from translation systems programmed with poly(A)$^+$ RNA isolated from normal rat liver (lane 1), from liver isolated from 3MC-treated rats (lane 2), from surrounding liver tissue isolated from 3MC-treated rats (lane 3), and nodular tissue isolated from 3MC-treated rats (lane 4). The arrow represents the migration of purified cytochrome P-448 on the $NaDodSO_4$/10% polyacrylamide gel. The molecular weight markers are identical to those in Fig. 1.

to define the molecular events that lead to alterations in the level and/or activity of various enzymes within the nodules. In the present study, we have found that DT-diaphorase mRNA as well as the Ya/Yc and Yb mRNAs of the glutathione transferases are increased in the nodules compared to surrounding liver tissue or normal tissue. In addition, the level of DT-diaphorase mRNA and the glutathione transferase Yb mRNA(s) can be augmented by 3MC administration, whereas the Ya/Yc mRNAs are no longer responsive to the inductive effect of 3MC. Cytochrome P-448 mRNA also appears to be induced normally in the nodules by 3MC treatment.

The increase in the various mRNA levels observed in this study is consistent with the increases found in the catalytic activity and/or level of glutathione transferases and DT-diaphorase in persistent hepatocyte nodules by other investigators (6, 10, 11). Consequently it would appear that the synthesis of these enzymes are increased specifically in the nodules as compared to the surrounding liver tissue and suggest a differential expression of the glutathione transferase and DT-diaphorase genes in nodular versus surrounding liver tissue during chemically induced neoplastic transformation.

Although the expression of genes encoding glutathione transferase and DT-diaphorase appears to be aberrant in persistent hepatocyte nodules, the normal regulatory mechanisms that lead to an induction of these mRNAs in normal tissue are for the most part still operative in nodular tissue. This is best illustrated by our observation that the levels of DT-diaphorase mRNA, cytochrome P-448 mRNA, and glutathione transferase Yb mRNA in nodular tissue are augmented by 3MC administration to rats.

In contrast, however, the glutathione transferase Ya/Yc mRNA levels in nodular tissue are not elevated by 3MC and suggest that the regulatory mechanisms that lead to an increase in these mRNAs are not functional in nodular tissue. Since the level of glutathione transferase Yb mRNA(s), DT-diaphorase mRNA, and cytochrome P-448 mRNA can be augmented in nodular tissue by 3MC, our data suggest that the lack of inducibility of the Ya/Yc mRNAs is a specific defect in the regulatory region of the gene(s) encoding this protein rather than any general defect in transcription or post-transcriptional processing. A detailed comparison of genes for the glutathione transferase Ya/Yc subunit in normal and nodular tissue should define the basis for the lack of induction of the Ya/Yc mRNAs in nodular tissue by 3MC.

One of the major questions regarding the changes in the various mRNA levels in nodular tissue is centered around the relationship these increased mRNA levels have to the carcinogenic process. Although this question cannot be answered presently, it is clear that a specific increase in the glutathione transferase and DT-diaphorase mRNAs (and their corresponding proteins) would confer added protection to cells against the cytotoxic effects of some carcinogens. As mentioned previously, Farber (2) has suggested that an early cellular event during liver carcinogenesis is the development of resistance in subpopulations of hepatocytes to the cytotoxic effects of carcinogens.

Although the molecular basis underlying the elevation of the mRNA levels in nodular tissue has not been defined in the present study, it is intriguing to speculate that the gene(s) encoding these drug metabolizing enzymes have been amplified in nodular tissue as a result of the acetylaminofluorene selection procedure utilized in the induction of the nodules. Amplification of specific genes has been found to be a typical response when mammalian cells are selected on the basis of their resistance to various xenobiotics (29–31).

In conclusion, an examination of the expression and organization of the glutathione transferase and DT-diaphorase genes in nodular tissue and the resulting hepatocellular carcinomas should provide important insight into regulatory mechanisms that are operative during chemically induced neoplastic transformation. The relationship these alterations have to the carcinogenic process will be an important question to address.

The expert technical assistance of Dianna Armstrong and Cathy Thompson in maintaining rats used in these studies is gratefully acknowledged. The authors would also like to thank Joan Kiliyanski for her assistance in the preparation of this manuscript. Part of the support for these studies was provided by the financial assistance of a Canadian Liver Foundation Grant and Scholarship to R.G.C.

1. Solt, D. & Farber, E. (1976) *Nature (London)* **263,** 701–703.
2. Farber, E. (1980) *Biochim. Biophys. Acta* **605,** 149–166.
3. Cameron, R., Sweeney, G. D., Jones, K., Lee, G. & Farber, E. (1976) *Cancer Res.* **36,** 3888–3893.
4. Okita, K., Noda, K., Fukumoto, Y. & Takemoto, T. (1976) *Gann* **67,** 899–902.
5. Griffin, M. J. & Kizer, D. E. (1978) *Cancer Res.* **38,** 1136–1141.
6. Schor, N. A., Ogawa, K., Lee, G. & Farber, E. (1978) *Cancer Lett.* **5,** 167–171.
7. Levin, W., Lu, A. Y. H., Thomas, P. E., Ryan, D., Kizer, D. & Griffin, M. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3240–3243.
8. Bentley, P., Waechter, F., Oesch, F. & Staubli, W. (1979) *Biochem. Biophys. Res. Commun.* **91,** 1101–1108.
9. Sharma, R. N., Gurtoo, H. L., Farber, E., Murray, R. K. & Cameron, R. G. (1981) *Cancer Res.* **41,** 3311–3319.
10. Kitahara, A., Satoh, K. & Sato, K. (1983) *Biochem. Biophys. Res. Commun.* **112,** 20–28.
11. Astrom, A., DePierre, J. W. & Eriksson, L. (1983) *Carcinogenesis (NY)* **4,** 577–581.
12. Farber, E., Parker, S. & Gruenstein, M. (1976) *Cancer Res.* **35,** 3279–3287.
13. Cayama, E., Tsuda, H., Sarma, D. S. R. & Farber, E. (1978) *Nature (London)* **275,** 60–62.
14. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
15. Pickett, C. B. & Lu, A. Y. H. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 893–897.
16. Huang, M.-T., Miwa, G. T. & Lu, A. Y. H. (1979) *J. Biol. Chem.* **254,** 3930–3934.
17. West, S. B., Huang, M. T., Miwa, G. T. & Lu, A. Y. H. (1979) *Arch. Biochem. Biophys.* **193,** 42–50.
18. Pickett, C. B., Jeter, R. L., Morin, J. & Lu, A. Y. H. (1981) *J. Biol. Chem.* **256,** 8815–8820.
19. Pickett, C. B., Telakowski-Hopkins, C. A., Donohue, A. M., Lu, A. Y. H. & Hales, B. F. (1982) *Biochem. Biophys. Res. Commun.* **104,** 611–619.
20. Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J.-F., Argenbright, L. & Lu, A. Y. H. (1984) *J. Biol. Chem.* **259,** 5182–5188.
21. Bailey, J. M. & Davidson, N. (1976) *Anal. Biochem.* **70,** 75–85.
22. Alwine, J. C., Kemp, D. J., Parker, B. A., Reiser, J., Renart, J., Stark, G. R. & Wahl, G. M. (1979) *Methods Enzymol.* **68,** 220–242.
23. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
24. Williams, J. B., Wang, R., Lu, A. Y. H. & Pickett, C. B. (1984) *Arch. Biochem. Biophys.* **232,** 408–413.
25. Bresnick, E., Brosseau, M., Levin, W., Reik, L., Ryan, D. E. & Thomas, P. E. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4083–4087.
26. Negishi, M. & Nebert, D. W. (1981) *J. Biol. Chem.* **256,** 6969–6974.
27. Fagan, J. B., Pastewka, J. V., Park, S. S., Guengerich, F. P. & Gelboin, H. V. (1982) *Biochemistry* **21,** 6574–6580.
28. Morville, A. L., Thomas, P., Levin, W., Reik, L., Ryan, D. E., Raphael, C. & Adesnik, M. (1983) *J. Biol. Chem.* **258,** 3901–3906.
29. Alt, F. W., Kellums, R. E., Bertins, J. R. & Schimke, R. T. (1978) *J. Biol. Chem.* **253,** 1357–1370.
30. Beach, L. R. & Palmiter, R. D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2110–2114.
31. Hunt, S. W. & Hoppe, P. A. (1983) *J. Biol. Chem.* **258,** 13185–13192.