Vol. 120, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 30, 1984 Pages 677-685

Tissue Specific Control of α-Fetoprotein Gene Expression

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Received March 27, 1984

The expression of the alpha-fetoprotein (AFP) gene was studied in rat liver and kidney. A significant level of AFP mRNA was found in neonatal liver and kidney, but not in adult tissues. Unlike liver the re-expression of AFP mRNA was not seen upon chemically induced regeneration of the kidney. Treatment of neonatal rats with dexamethasone caused a decrease in liver AFP mRNA levels, but a similar decrease was not apparent in kidney. Northern analysis revealed AFP mRNA size to be identical in neonatal liver and kidney. The results suggest different gene regulatory mechanisms in liver and kidney for the AFP gene.

Alpha-fetoprotein (AFP) has served as an useful model in the understanding of gene regulation during development and carcinogenesis (1,2). AFP is a serum protein produced at high levels in the fetal tissues: liver and yolk sac (1,3). Shortly after birth serum AFP levels decrease dramatically as a result of decreased message levels (4-9). By 4 weeks AFP mRNA exists at an almost undetectable level which remains throughout adulthood. The AFP mRNA level can be lowered prematurely by a single injection of the synthetic glucocorticoid dexamethasone (10,11). This decrease in message is clearly visible within a few hours following injection and continues to decrease as existing message degrades (11,12).

The low levels of AFP mRNA that exist in adult liver can be altered in certain abnormal states. AFP mRNA level has been found to be elevated in regenerating liver (13), as well as certain hepatocellular carcinogenic states (8,14). This elevated message level is also responsive to dexamethasone suppression (15). It is therefore possible through combined study to investi-

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0006-291X/84 $1.50

gate the activation as well as the inactivation of transcriptional control of AFP in rat liver.

In addition to liver and yolk sac there has been a report of AFP mRNA in developing kidney (9). Unlike liver and yolk sac which are endodermal in origin, the kidneys are derived from the mesodermal layer during development. It would therefore be of interest to ascertain whether regulation of AFP mRNA in kidney occurs in a manner similar to that seen in liver. In this paper we looked at repression and derepression of AFP gene expression during kidney development and regeneration. The depression of AFP gene expression in kidney and liver was also compared by its response to glucocorticoid hormone. Our results indicate that regulation of AFP gene expression in kidney may be controlled by a mechanism different from that found in liver.

Materials and Methods

Animals. Kidney regeneration was induced in Sprague-Dawley rats (40-60g), which were fasted for 12 hrs, by a single intraperitoneal injection with 35 mg/ml folic acid in 0.154 M $NaHCO_3$, pH 7.0 according to Threlfall et al. (16). An effective dose of 20 ml/kg body weight was used. Animals were sacrificed at designated time points and kidneys quickly removed and weighed prior to RNA isolation.

A single dose of dexamethasone (Elkins-Sinn, Inc., Cherry Hill, N.J.) (2 µg/gm) was injected intraperitoneally into 2 day old Sprague-Dawley rat pups. Uninjected litter mates served as controls. Newborn rats were sacrificed 24 hours following injection and liver and kidneys removed for RNA isolation.

RNA Isolation. Total RNA was isolated as previously described by Chirgwin et al., (17) with modification. Tissue was homogenized in 10 volumes (w/v), 7.5 M guanidine-HCl, 25 mM Na-Citrate, 1% 2-mercaptoethanol, pH 7.0 at 0°C for 30-60 seconds at full speed with a polytron homogenizer (Brinkman Instruments). Cellular debris was removed by centrifugation for 5 min at 800 x g, 0°C. The supernatant was layered over 1/3 volume of 5.7 M CsCl and centrifuged at 150K x g for 16 hrs at 20°C. The RNA pellet was washed with cold 70% ethanol, dried, dissolved in sterile H_2O, and stored at -70°C until use. In some cases poly(A^+)RNA was isolated from total RNA by passage twice through an oligo(dT) cellulose column as described by Aviv and Leder (18).

Dot Hybridization. Dot hybridization followed the method of Kafatos et al. (19). RNA samples in 2X SSC (SSC = 0.15 M NaCl, 0.015 M Na-Citrate), were spotted onto a nitrocellulose filter previously saturated with 20X SSC using a Minifold™ (Schleicher and Schuell) filtration manifold. Samples were washed with 20X SSC, the nitrocellulose filter allowed to dry at room temperature and then baked at 80°C for 2 hrs. The prehybridization, hybridization and washing were identical to those used by Thomas (20). The hybridization solution contained approximately 1 x 10^6 cpm/ml of nick translated cloned AFP cDNA pRAF87 of specific activity 1-3 x 10^8 cpm/µg. Filters were autoradiographed using Kodak XAR-5 X-ray film and a Dupont Cronex™ intensifying screen. Following autoradiography, dots were cut from the nitrocellulose filter and

dissolved in Filtron-X™ (National Diagnostics) for scintillation counting. An area not containing RNA served to determine background counts.

Northern Analysis. For Northern gel studies, poly(A^+)RNA samples were run on formaldehyde-agarose denaturing gels as described by Lehrah et al. (21), using an identical buffering system. Following electrophoresis, gels were used untreated for transfer of RNA to nitrocellulose filters. Gels were transferred using 20X SSC as the transfer solution as previously described (20). Prehybridization, hybridization and washing procedures were as above (20).

Results and Discussion:

Tissue specific production of AFP during development is known but not well documented for all tissues. Many studies have focused on liver and yolk sac, as the major contributors to serum AFP in fetal and neonatal animals (1-3). The reports of AFP mRNA in newborn kidney (9), as well as AFP production in cultured kidney cells (22), prompted us to look at the regulation of AFP mRNA in kidney.

The presence of AFP mRNA in newborn kidney was confirmed by dot hybridization (Fig. 1). Positive hybridization was seen with kidney RNA isolated

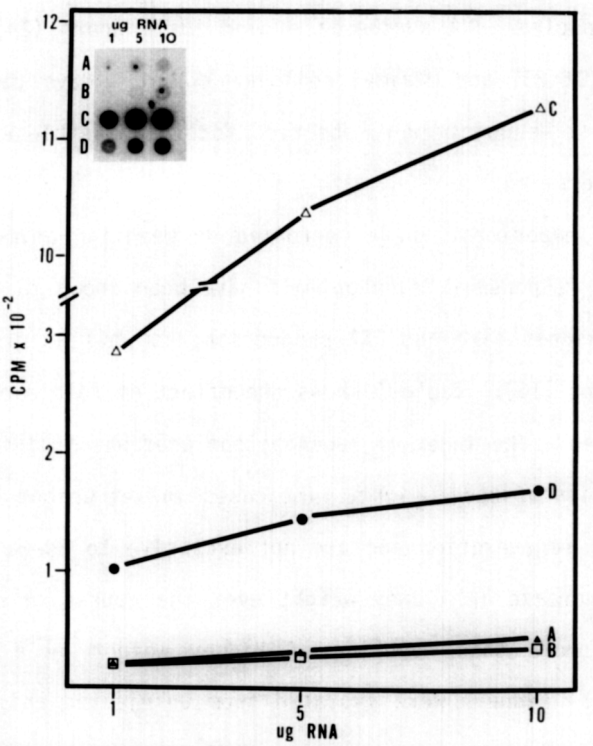

Figure 1. Age dependent AFP mRNA expression in rat liver and kidney. Autoradiograph and quantification of dot hybridization of [^{32}P]labeled AFP cDNA probe to total RNA prepared from adult liver (A), adult kidney (B), 4 day old liver (C), and 4 day old rat kidney (D). After autoradiography, each dot was cut out and counted in a liquid scintillation counter for quantification.

from 4 day old rats to an extent lesser than that seen in 4 day old rat liver, but considerably greater than that seen in adult liver or kidney. These reports agree with previously reported data (9). The depression of AFP mRNA with development in kidney is identical to that seen in liver (9), suggesting a similar mechanism of AFP gene regulation. If the mechanism of AFP gene regulation is indeed similar between kidney and liver, we should expect to see the same pattern of activation and inactivation of AFP gene in kidney as seen in liver.

The derepression of AFP mRNA has been demonstrated in regenerating liver as a result of chemical injury (13). The administration of CCl_4 to adult rats has been shown to result in a 10-fold increase in AFP mRNA (13). Chemically induced regeneration of kidney has been described previously (16,23). A single intravenous or intraperitoneal injection of folic acid has been shown to result in a permanent hypertrophy of the kidney (16). This regeneration results in peak nuclear RNA synthesis around 10-12 hours (24), peak mitotic index at 2 days (16,23) and maximal cellular RNA at 4 days (16,23) following injection. These time points were observed for the possible elevation of AFP mRNA during regeneration.

Regeneration experiments were performed on weanling animals (40-60g) to provide a maximum response. Young animals have been shown to give the greatest increase in kidney size and RNA production, as well as greater synchrony in mitotic labeling (16). Table 1 shows the effect of folate on kidney weight in the animals used. The index of regeneration used was individual wet kidney weight as a function of body weight. Increases in wet weight have been shown to correlate with regeneration and are not exclusive to edema (16). Table 1 shows a steady increase in kidney weight over the course of the experiment. The result is a more than doubling of kidney weight by 4 days following injection. Control kidneys were isolated from uninjected animals on the 4th day of the experiment.

Total RNA was isolated from folate treated kidneys and levels of AFP mRNA measured by dot hybridization (Fig. 2). As seen in Figure 2 no AFP message

Table 1: The Effect of Folic Acid Treatment on Rat Kidney Weight

Group	mg/gm*
Control	5.20 ± 0.25
10 hour	9.49 ± 0.64
2 day	12.00 ± 0.64
4 day	13.87 ± 0.88

*Milligram kidney weight per gram body weight ± standard error.

was detectable by this method at any time point. This result is unlike the 10-fold increase in AFP mRNA observed in regenerating liver (13). The results suggest that increased cell proliferation in kidney though causing an increase in cellular RNA content (our results not shown) (16,23), does not result in AFP mRNA specific deregulation.

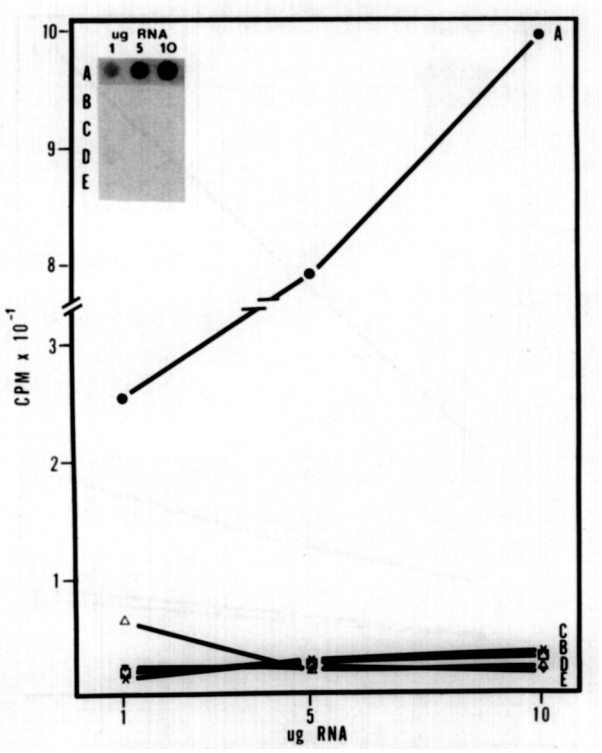

Figure 2. Effect of kidney regeneration on AFP mRNA expression. Autoradiography and quantification of dot hybridization of [^{32}P]labeled AFP cDNA probe to total RNA prepared from 4 day old rat kidney (A), and weanling rat kidney (control, B), 10 hours (C), 2 days (D), and 4 days (E) following folate injection.

The expression of AFP mRNA in developing liver can be suppressed prematurely by a single injection of the synthetic glucocorticoid dexamethasone. The efficacy of message suppression is quite pronounced in liver resulting in a 50-fold reduction in AFP mRNA by 4 days following injection of dexamethasone (2 µg/gm) (10,11). A depression of kidney AFP mRNA should therefore be easily detected if similar mechanisms of regulation exist.

Dot hybridization was performed on total RNA isolated from the livers and kidneys of animals treated or untreated with dexamethasone (Fig. 3). Dexamethasone is seen to cause a marked depression of AFP mRNA in liver, but no affect on kidney AFP mRNA levels. This absence of a response is not due to the lack of dexamethasone receptors in kidney (25), or an inability of receptor complex to bring about a change in mRNA pools (26).

Recently various sizes of AFP and AFP mRNA were demonstrated in adult differentiated liver cells (27,28). The apparent differences in the regula-

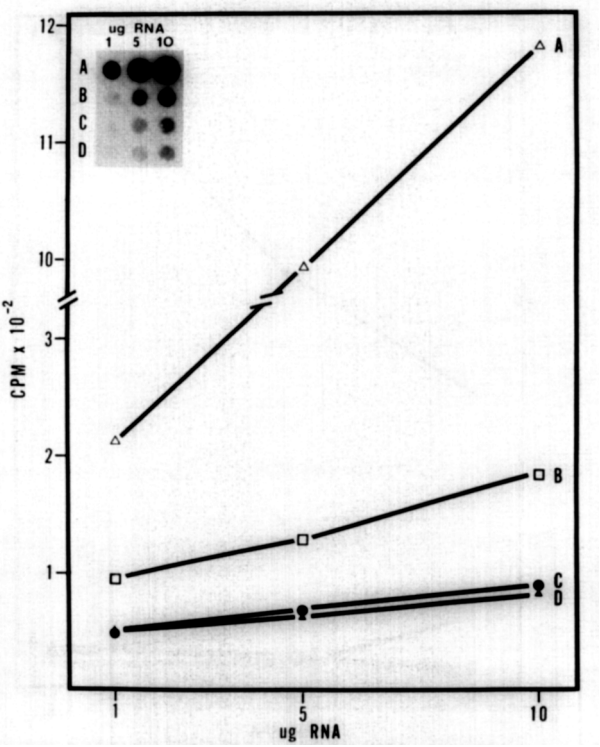

Figure 3. The effect of dexamethasone on AFP mRNA expression. Autoradiograph and quantification of dot hybridization of [^{32}P]labeled AFP cDNA probe to total RNA prepared from liver (A) and kidney (C) of 3 day old control rats, and from liver (B) and kidney (D) of 3 day old rats treated with a single injection of dexamethasone (2 µg/g) for 24 hours.

tion of the AFP gene in liver and kidney prompted us to look at the AFP mRNA size. The differences seen between the two tissues might be the result of separate mRNA species. Northern analysis of neonatal liver and kidney poly-(A^+)RNA revealed prominent bands at 2.1 kb in both tissues (Fig. 4). This result supports the existence of single common mRNA species in neonatal liver and kidney.

The results of this paper are supportive of an alternative mechanism of AFP mRNA regulation in kidney as those seen in liver. This belief is supported by evidence that nondevelopmentally regulated proteins of liver and kidney show unrelated elevation and depression after dexamethasone treatment as judged by two-dimensional electrophoresis (26). One possible mechanism for this would be the existance of heterogenous glucocorticoid receptors. The

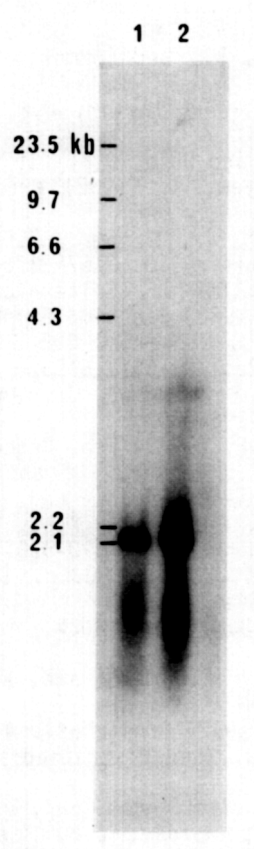

Figure 4. Northern analysis of AFP mRNA. Poly(A^+)RNA was isolated from 4 day old rat kidney and liver. Lane 1 contains 0.5 µg of liver poly(A^+)RNA, Lane 2 contains 15 µg of kidney poly(A^+)RNA. E. coli RNA was added to the liver poly(A^+)RNA sample to provide equal amounts of RNA in each lane.

kidney has in fact been shown to contain a prominent glucocorticoid receptor greatly reduced in liver, and the liver a receptor greatly reduced in the kidney (29,30). The reason for two separate mechanisms of regulation for the same gene is unclear. The difference in germ layer origin is a strong possibility that needs to be investigated further.

Acknowledgements

This investigation was supported by USPHS Grant CA 25098 awarded by NCI. G.J. Cote is a trainee of Cancer Biology Training Grant T32-09286. The plasmid pRAF87 was a generous gift of Dr. Thomas Sargent. We also thank Mr. Robert Shaw for assistance in preparing this manuscript.

References

1. Abelev, G.I. (1971) Advan. Cancer Res. 14, 295-357.
2. Becker, F.F., and Sell, S. (1974) Cancer Res. 34, 2489-2494.
3. Adinolfi, M. (1979) Adv. Hum. Gen. 9, 165-228.
4. Chiu, J.F., Decha-Umphai, W., and Commer, P. (1979) Nucl. Acids Res. 7, 239-249.
5. Liao, W.S.L., Conn, A.R. and Taylor, J.M. (1980) J. Biol. Chem. 255, 10036-10039.
6. Belanger, L., Commer, P. and Chiu, J.F. (1979) Cancer Res. 39, 2141-2148.
7. Tilghman, S.M. and Belayew, A. (1982) Proc. Natl. Acad. Sci. USA 79, 5254-5257.
8. Sala-Trepat, J.M., Dever, J., Sargent, T.D., Thomas, K., Sell, S. and Bonner, J. (1979) Biochemistry 18, 2167-2178.
9. Selten, G.C.M., Princen, H.M.G., Selten-Versteegen, A.M.E., Mol-Backx, G.P.B.M., and Yap, S.H. (1982) Biochim. Biophys. Acta 699, 131-137.
10. Chiu, J.F., Massari, R.J., Schwartz, C.E., Meisler, N.T., and Thanassi, J.W. (1981) Nucl. Acids Res. 9, 6917-6933.
11. Guertin, M., Baril, P., Bartkowiak, J., Anderson, A. and Belanger, L. (1983) Biochemistry 22, 4296-4302.
12. Massari, R., and Chiu, J.F. (1982) Fed. Proceed. 41, 681.
13. Chiu, J.F., Gabryelak, T., Commer, P., and Massari, R. (1981) Biochem. Biophys. Res. Commun. 98, 250-254.
14. Chiu, J.F., Huang, D.P., Burkhardt, A.L., Cote, G. and Schwartz, C.E. (1983) Arch. Biochem. Biophys. 222, 310-320.
15. Schwartz, C.E., Burkhardt, A.L., Huang, D.P., and Chiu, J.F. (1982) Biosci. Reports 2, 777-784.
16. Threlfall, G., Taylor, D.M., and Buck, A.T. (1966) Lab. Invest. 15, 1477-1485.
17. Chirgwin, J.M., Przbyla, A.E., MacDonald, R.J., and Rutter, W.J. (1979) Biochemistry 18, 5294-5299.
18. Aviv, H., and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 77, 5201-5205.
19. Kafatos, F.C., Jones, C.W., and Efstratiadis, A. (1979) Nucl. Acids Res. 7, 1541-1552.
20. Thomas, P.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5201-5205.
21. Lehrah, H., Diamond, D., Wozney, J.M., and Boedtker, H. (1977) Biochemistry 16, 4743-4751.
22. Gitlin, D., Pericelli, A. and Gitlin, C.M. (1972) Cancer Res. 32, 979-982.
23. Baserga, R., Thatcher, D., and Marzi, D. (1968) Lab. Invest. 19, 92-96.

24. Brade, W.P., Thomson, J.A., Chiu, J.F., and Hnilica, L.S. (1974) Exp. Cell Res. 84, 183-190.
25. Funder, J.W., Feldman, D., and Edelman, I.S. (1973) Endocrinology 92, 1005-1013.
26. Iynedjian, P.B., and Jacot, M.M. (1981) J. Biol. Chem. 256, 7069-7076.
27. Chou, J.Y., Ito, F., Evans, G., Chiu, J.F. and Feldman, M. (1982) Biochem. Biophys. Res. Commun. 108, 1524-1530.
28. Gal, A., Nahon, J. and Sala-Trepat, J.M. (1983) Anal. Biochem. 132, 190-194.
29. Litwack, G. and Rosenfield, S.A. (1975) J. Biol. Chem. 250, 6799-6805.
30. Markovic, R.D., Eisen, H.J., Parchman, L.G., Barnett, C.A. and Litwack, L.G. (1980) Biochemistry 19, 4556-4564.