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EXPRESSION OF ALPHA-FETOPROTEIN AND ALBUMIN GENES IN A RAT HEPATOMA CELL LINE SY/1/80: EVIDENCE FOR THE NEED OF SPECIES SPECIFIC SERUM FACTORS

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Summary

The expression of alpha-fetoprotein and albumin genes has been studied in a rat hepatoma cell line (SY/1/80) developed from a liver cell tumor induced with diethylnitrosamine. This original tumor produces both proteins. However, in in vitro propagated hepatoma cells, after passages in growth medium containing new born calf serum, the mRNAs of both proteins were undetectable. Supplementation with rat serum, but not serum from calf, horse or human, in growth medium for this cell line led to resynthesis of albumin and AFPmRNAs. These findings suggest that species specific serum factor(s) play an important role in the regulation of gene expression. Although the nature of the factor(s) and of the mechanism of action remains to be elucidated, this phenomenon may explain the general feature of diminishing abilities of cells to produce specific proteins in continuous subculture using standard calf serum.

Introduction

Albumin is a specific plasma protein synthesized in the liver. The synthesis of albumin is initiated before birth and completely activated after birth. In hepatoma cells, albumin synthesis can be reduced or abolished (1,2). Alpha-fetoprotein (AFP) is a serum alpha-1-globulin synthesized during ontogenesis primarily in the yolk sac and fetal liver (3) and neosynthesized during carcinogenesis in hepatocellular carcinoma. The biologic function of AFP is still unknown. The amino acid sequence homology between albumin and AFP, and the cross-immune reactions of breakdown products (4) suggest that these two proteins are derived from the same ancestral gene. Furthermore, the similarity in physical and chemical properties and the fact that AFP biosynthesis diminishes in the perinatal period as albumin synthesis and serum concentration rise, indicate that the protein may have a function similar to that of albumin. The neosynthesis of AFP during oncogenesis is understood as a derepression of the AFP gene normally repressed during adult life. Understanding of the mechanisms that control the expression of the albumin and AFP genes in vivo or in vitro may, therefore, provide insight into the molecular basis of ontogenic development and malignant transformation. We now present evidence in a rat hepatoma cell line (SY/1/80) for the need of rat serum factor(s) to express the AFP and albumin genes. In

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these experiments, the utilization of serum obtained from calf, horse or human instead of rat serum in growth medium for six weeks, failed to de-repress the AFP and albumin genes. These findings suggest that species specific extracellular (serum) factors play an important role in the regulation of expression of the genes coding for these proteins.

Materials and methods

Sprague Dawley rats were used thoughout and were maintained on standard Purina Chow and water ad libitum until sacrificed. Embryonic and postnatal organs or tissues were isolated at different stages of development. The hepatomas were induced in male Sprague Dawley rats by feeding of 60 ppm of diethylnitrosamine in drinking water (5).
Antibodies of goat anti rat serum albumin and of goat anti rat serum AFP were prepared and made RNase free as previously described (6).
The preparation of total polyribosomes from fetal and neonatal liver, kidney, brain and diethylnitrosamine induced hepatomas were performed according to the method described by Schimke et al (7). Polyribosomal RNA from the isolated polyribosomes and poly A^+RNA were prepared as described previously (8).
For the preparation of total cytoplasmic RNA from hepatoma cells, cells were homogenized in 50 mM Tris-HCI pH 7.0 containing: 5 mM $MgCl_2$, 25 mM KCl, 0.3% deoxycholate, 0.2% Nonidet P-40, 0.25 M sucrose and 3 mM glutathione. After centrifugation at 9000 x g for 10 min., total RNA was isolated from the supernatant as described previously (8).
Alpha-fetoprotein (AFP)mRNA was purified from total yolk sac polyribosomes according to the method described for the isolation of albumin mRNA (8,9). The isolated mRNAs were transcribed into (^{3}H)-cDNA under conditions reported previously (8). However, the synthesized (^{3}H)-cDNA from AFPmRNA had to be purified as reported by Liao et al (10) because of the presence of non-hybridizable material.
(^{3}H)-Labelled cell-free translation products made under the direction of poly A^+RNAs and purified mRNAs were examined on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as described by Laemmli (11). Autoradiograms were obtained by exposure to X-ray film after treatment of the gels as described by Bonner and Laskey (12).
Analytical RNA-cDNA hybridization were performed according to the method of Housman et al (13) as previously described (8).

Results and discussion

Recently, we have purified mRNAs of albumin and AFP from rat (Sprague Dawley) liver and yolk sac respectively. Purification of albumin mRNA was performed using the method previously described (6,8) except that goat anti-rat albumin gamma globulin was used as first antibody followed by precipitation of immune-complexes with rabbit anti-goat gamma globulin. Translation of poly A containing RNA prepared from the immunoprecipitated polyribosomes in a wheat germ cell-free system and sequence complexity analysis of this RNA fraction with the complementary DNA transcribed from this RNA show that the purified RNA represents albumin mRNA (fig. 1 and fig. 2). AFPmRNA was purified from total polyribosomes of yolk sac according to the method used for the isolation of albumin mRNA. As shown in fig.1 and fig.2, the mRNA iso-

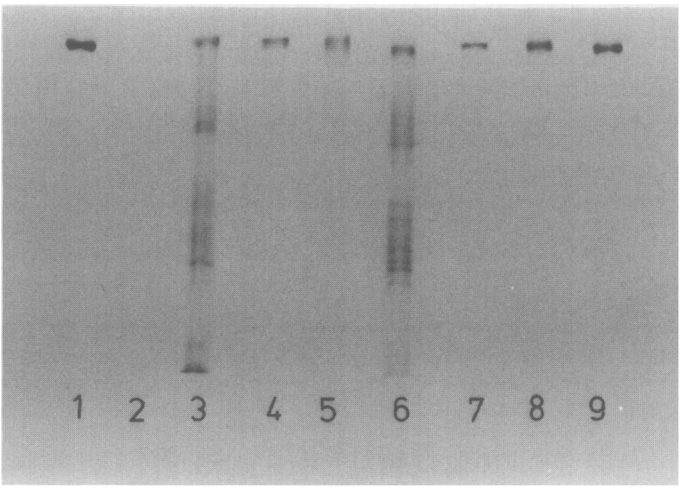

Figure 1. $NaDodSO_4$/polyacrylamide slab gel electrophoresis of cell-free translation products.
Various RNA preparations were translated in a wheat germ system and radioactively labelled products synthesized in vitro were analysed electrophoretically on 10% polyacrylamide/$NaDodSO_4$ slab gel. Lanes 1 and 9: rat serum (^{14}C)-albumin marker. Lane 2: wheat germ extract to which no exogenous RNA was added. Lane 3: cell-free products synthesized under the direction of total cytoplasmic polyadenylylated RNA prepared from yolk sac. Lane 6: cell-free products synthesized under the direction of total cytoplasmic polyadenylylated RNA prepared from adult rat liver. Lanes 4 and 7: cell-free products synthesized under the direction of AFP and albumin mRNA respectively. Lane 5: immuno-precipitated material from cell-free products synthesized under the direction of AFPmRNA with anti AFP antiserum. Lane 8: immuno-precipitated material from cell-free products synthesized under the direction of albumin mRNA with anti rat serum albumin antiserum. (Due to photographical reason, the upper part of the autoradiograph has been left out).

lated had been purified to homogeneity as demonstrated by a wheat germ translation system and by hybridization kinetic analysis respectively. No cross-annealings were found, when albumin mRNA and AFPcDNA or AFPmRNA and albumin cDNA were hybridized (data not shown).

Using molecular hybridization technology we have quantitated the AFP- and albumin mRNA content of serveral organs and tisues of Sprague Dawley rats during fetal and neonatal development. Our results indicate that the concentration of AFPmRNA sequences in RNA fractions extracted from yolk sac varies and depends on the gestational age. In contrast, albumin mRNA is barely detected in yolk sac. A representative result is demonstrated in figure 2. This finding is consistent with that of other investigators (10,14). Although both AFP and albumin mRNA could be detected in fetal and neonatal developing kidneys, the results show the absence of AFP- and albumin mRNA sequences in the kidney of adult animals. In brain, there were no detec-

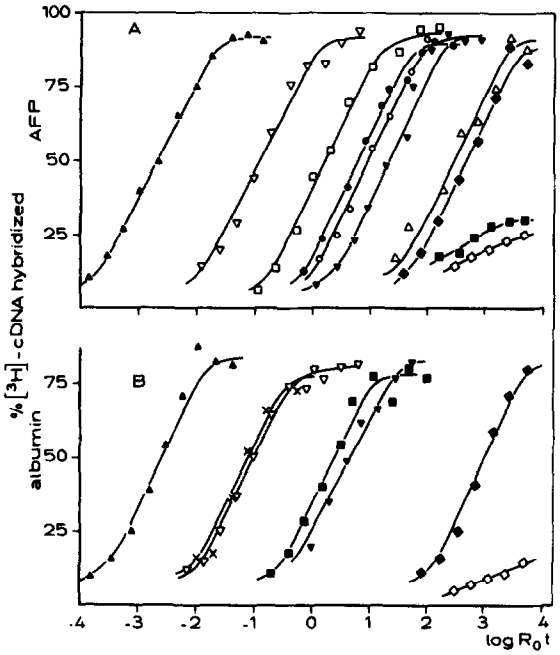

Figure 2. Hybridization analysis of various RNA fractions prepared from several tissues of rats during fetal and postnatal development and RNA fractions prepared from hepatomas induced with diethylnitrosamine.
(A) AFPcDNA was hybridized to the RNA from which it was transcribed (▲); to the poly A containing RNA prepared from hepatomas induced with diethylnitrosamine (▼); to total RNAs extracted from 19 day old yolk sac (▣); from livers of two week old rats (●); from 14 day fetal livers (○); from hepatomas induced with diethylnitrosamine (▼); from livers of 3 week old rats (▲); from kidneys of 10 day old rats (◆); from adult rat livers (■); or to the RNA prepared from adult rat kidneys (◆).
(B) Albumine cDNA was hybridized to the RNA from which it was transcribed (▲); to poly A containing RNA prepared from adult rat liver (✖) and prepared from hepatomas induced with diethylnitrosamine (▼); to total RNAs extracted from adult rat livers (■); extracted from hepatomas induced with diethylnitrosamine (▼); to total RNA extracted from kidneys of 10 day old rats (◆) or to total RNA prepared from adult rat kidneys (◆).

table AFP- and albumin mRNA sequences during fetal and neonatal life even when hybridization reactions were carried to significantly high R_0t values (data not shown). The AFPmRNA concentration in RNA fractions extracted from fetal liver and liver shortly after birth was relatively constant. However, the concentration decreased significantly in 21 day old neonatal liver and was barely detectable in adult liver. In experimental hepatomas of rats induced with diethylnitrosamine, the AFPmRNA concentration is elevated to the level of fetal liver (fig. 2). However, the concentration of albumin mRNA did not change during the oncogenesis.

In order to study the mechanism of repression and derepression of AFP- and albumin genes more directly, we have developed a rat hepatoma cell line from

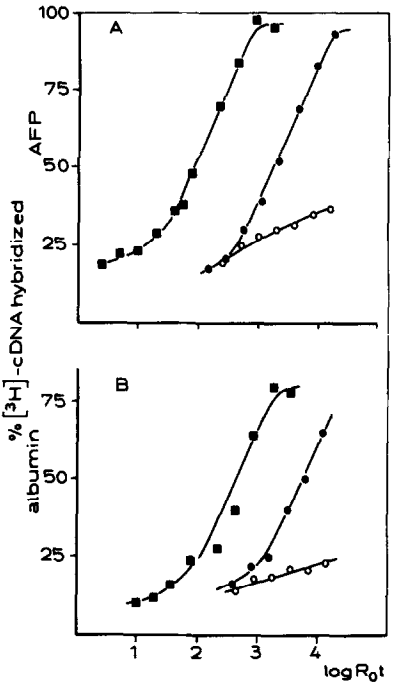

Figure 3. Hybridization analysis of various RNA fractions prepared from rat hepatoma cell line SY/1/80 grown in medium obtained from different species. (A) AFPcDNA was hybridized to the total RNA prepared from hepatoma cells grown for 6 weeks in medium containing 10% new born calf serum (o) or 10% adult rat serum (●) or to the polyadenylylated RNA prepared from hepatoma cells grown for 6 weeks in medium containing 10% adult rat serum (■).
(B) Albumin cDNA was hybridized to the total RNA prepared from hepatoma cells grown for 6 weeks in medium containing 10% new born calf serum (o) or 10% adult rat serum (●) or to poly A containing RNA prepared from cells grown for 6 weeks in medium containing 10% adult rat serum (■).
Hybridization analysis of RNA fractions prepared from cells of this cell line grown for 6 weeks in medium containing 20% fetal calf serum; 10% human or 10% horse serum and grown for 2 and 4 weeks in medium containing 10% adult rat serum gave similar findings as the hybridization analysis of RNA fraction prepared from cells grown in medium containing 10% new born calf serum (o).

liver cell carcinoma induced with diethylnitrosamine. The original tumor produced AFP and albumin. The hybridization kinetic analysis to measure the sequence contents of AFP- and albumin mRNAs in this tumor are shown in fig. 2. The cells isolated from the tumor were grown in BMEM (basal minimal essential medium), containing vitamins and antibiotics, at 37 °C and during the primary passages 20% (v/v) of fetal calf serum was added. After the primary passages, 10% (v/v) of new born calfs serum was utilized. As shown in figure 3 there is no AFPmRNA nor albumin mRNA detectable in RNA fraction prepared from these tumor cells propagated in vitro. The same results were obtained

from the tumor induced by inoculation of these cells (SY/1/80) into nude mice after a sublethal exposure to radiation (data not shown).

In in vitro propagated hepatoma cells, it has been shown that the expression of specific proteins e.g. albumin appears to be transient and may either cease or become greatly diminished with continuous subcultures (15,16). To examine whether the use of species specific serum in growth medium would change the transcription pattern of cells in our line, 10% of adult rat serum was utilized in growth medium instead of new born calf serum. For routine maintainance, part of these cells were still grown in medium supplemented with 10% new born calf serum. There were no apparent differences in the growth rate and in the light microscopic aspects of these cells when the medium was supplemented with adult rat serum. The hybridization analysis of RNA prepared from these cells grown for 6 weeks in this medium, however, shows evidence of the presence of significant amount of AFP and albumin mRNA (fig.3). These mRNAs were not detectable in RNA fraction prepared from these cells after only 2 or 4 weeks of culture in medium containing rat serum. These results were consistent in subsequent experiments starting from the cells grown in medium supplemented with 10% new born calf serum. Furthermore, supplementation of 20% fetal calf serum, 10% of horse or human serum failed to induce the synthesis of AFP- and albumin mRNAs in this cell line grown for 6 weeks in parellel experiments (fig.3).

Although many studies have shown that hormones e.g. glucocorticosteroids and analogs of cAMP mediate increases in the synthesis and secretion of hepatic proteins including albumin in vivo or on cells in culture (17) and many studies had been done on the growth factors of cells in vitro including epidermal growth factor (18), there is no report concerning factor of factors involving the genomic de-repression in hepatic cell cultures. Recently, Higgens and Borenfreund (19) reported that heterotransplantation of several long term rat liver cell lines into nude mice and subsequent re-establishment in vitro resulted in hepatocyte cultures expressing both alpha-fetoprotein and albumin. However, our experiments using this hepatoma cell line (SY/1/80) grown in nude mice as mentioned before failed to induce the resynthesis of mRNAs of both proteins. In contrast, supplementation of rat serum but not serum from calf, horse or human leads to an induction of synthesis of albumin and AFPmRNAs. It seems therefore that this SY/1/80 cell line is dependent on the species specific serum factor or factors for the synthesis of specific mRNAs. Whether this mechanism involves only the AFP and albumin genes, or other genes as well, and whether this phenomenon will be found in other cell lines are still unclear. Further studies of the nature of the factor(s) and of the mechanism of action are in progress.

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References

1. Schreiber, G., Roterrmund, H.M., Maeno, H., Weigand, K. and Lesch, R. (1969) Eur. J. Biochem. 10, 355-361.
2. Sala-Trepat, J.M., Dever, J., Sargent, D., Thomas, K., Sell, S. and Bonner, J. (1979) Biochemistry 18, 2167-2178.
3. Sell, S. and Becker, F.F. (1978) J. Natl. Canc. Inst. 60, 19-26.
4. Ruoslahti, E. and Engvall, E. (1976) Proc. Natl. Acad. Sci. USA 73, 4641-4644.
5. Becker, F.F. and Sell, S. (1979) Cancer Res. 39, 1437-1442.
6. Yap, S.H., Strair, R.K. and Shafritz, D.A. (1978) J. Biol. Chem. 253, 4944-4950.
7. Schimke, R.T., Palacios, R., Sullivan, D., Kiely, M.L., Gonzales, C. and Taylor, J.M. (1974) Meth. of Enzymology 30, 631-648.
8. Strair, R.K., Yap, S.H. and Shafritz, D.A. (1977) Proc. Natl. Acad. Sci. USA 74, 4346-4350.
9. Yap, S.H., Strair, R.K. and Shafritz, D.A. (1977) Proc. Natl. Acad. Sci. USA 74, 5397-5401.
10. Liao, W.S.L., Conn, A.R. and Taylor, J.M. (1980) J. Biol. Chem. 255, 10036-10039.
11. Laemmli, U.K. (1970) Nature 227, 680-685.
12. Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83-88.
13. Housman, D., Skoultchi, A., Forget, B.G. and Benz, E.J. (1974) Ann. N.Y. Acad. Sci. 241, 280-289.
14. Sell, S., Sala-Trepat, J.M., Sargent, T.D., Thomas, K., Nahon, J.L., Gootman, T.A. and Bonner, J. (1980) Cell Biology International Reports 4 (3), 235-254.
15. Borenfreund, E., Higgens, P.J., Steinglass, M. and Bendich, A. (1979) Cancer Res. 39, 800-807.
16. Irlin, I.S., Perova, S.D. and Abelev, G.I. (1966) Int. J. Canc. 1, 337-347.
17. Brown, P.C. and Papaconstantinous, J. (1979) J. Biol. Chem. 254, 9379-9384.
18. Kaneko, Y., Imai, Y., Matsuzaki, F., Endo, Y. and Ota, T. (1979) Experentia 35, 1660-1661.
19. Higgens, P.J. and Borenfreund, E. (1981) Exp. Cell. Biol. 49, 68-77.