# Sequence content of α-fetoprotein, albumin and fibrinogen polypeptide mRNAs in different organs, developing tissues and in liver during carcinogenesis in rats

**ARTICLE** *in* BIOCHIMICA ET BIOPHYSICA ACTA · DECEMBER 1982

Impact Factor: 4.66 · DOI: 10.1016/0167-4781(82)90146-4 · Source: PubMed

*Biochimica et Biophysica Acta*, 699 (1982) 131–137
Elsevier Biomedical Press



BBA 91136

# SEQUENCE CONTENT OF α-FETOPROTEIN, ALBUMIN AND FIBRINOGEN POLYPEPTIDE mRNAs IN DIFFERENT ORGANS, DEVELOPING TISSUES AND IN LIVER DURING CARCINOGENESIS IN RATS

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(Received May 5th, 1982)

*Key words: α-Fetoprotein synthesis; Albumin synthesis; Fibrinogen synthesis; mRNA sequence; Carcino genesis; (Rat)*

**To investigate the variable gene activities of α-fetoprotein, albumin and fibrinogen polypeptides as markers of 'liver specific proteins' in different developing organs or tissues, we have used specific complementary DNA probes to detect and to quantitate α-fetoprotein, albumin and fibrinogen polypeptide mRNA, respectively, in RNA fractions, prepared from various tissues of rats at different stages of fetal and postnatal development and from hepatomas induced by diethylnitrosamine. The results indicate that there is no consistent relationship between sequence content of α-fetoprotein, albumin and fibrinogen polypeptide mRNA in different developing tissues. Intestines which are like the liver also of endodermal origin do not contain α-fetoprotein, albumin and fibrinogen polypeptide mRNAs, while kidneys which are mesodermal in origin were found to be α-fetoprotein, albumin and fibrinogen polypeptide mRNA producers in neonatal life. In yolk sac, only α-fetoprotein and fibrinogen polypeptide mRNA could be detected. In the liver, the increased level of albumin and fibrinogen polypeptide mRNA during fetal and neonatal development is accompanied with a diminished amount of α-fetoprotein mRNA. The neosynthesis of α-fetoprotein mRNA in the liver during carcinogenesis occurred without a decreased content of albumin and fibrinogen polypeptide mRNAs. These findings suggest that complex mechanisms of gene regulation are involved in variable gene activities of α-fetoprotein, albumin and fibrinogen polypeptides in cells of different organs or tissues developed from a single cell.**

## Introduction

For a better understanding of the control mechanisms for the synthesis of 'liver specific' proteins such as albumin and fibrinogen and for the resynthesis of oncofetal protein in the liver such as α-fetoprotein, it is important to know the extrahepatic site or sites of synthesis of these proteins during fetal and postnatal development. After the discovery of α-fetoprotein in fetal calf serum by Pederson in 1944 [1] and in transplantable mouse hepatomas in 1963 by Abelev et al. [2], there were several studies reporting the sites of α-fetoprotein synthesis at different stages of fetal and neonatal development. The α-fetoprotein production has been found in significant quantities in fetal liver, yolk sac and in hepatocellular carcinoma [4–9] and in trace amounts in intestine, stomach and brain [10–12]. Most of the studies, however,

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Abbreviations: $R_o t$, product of initial RNA concentration in mol nucleotides/l and time in s (assuming that $A_{260}$ of 1.0 corresponds to 40 μg RNA/ml); $R_o t_{1/2}$, the $R_o t$ value at 50% hybridization; SDS, sodium dodecyl sulphate;

presented evidences using immunological or immunocytochemical techniques. The cellular uptake of plasma protein can therefore not be differentiated with a cellular synthesis site. Information concerning synthesis sites for albumin and for fibrinogen during embryonal development was scarce [13,14]. In this study, we have used specific complementary DNA probes to detect and to quantitate α-fetoprotein mRNA, fibrinogen polypeptide mRNA and albumin mRNA, respectively, in RNA fractions prepared from various tissues of rats at different stages of fetal and postnatal development and from hepatomas. Our results indicate that there is no consistent relationship between sequence content of α-fetoprotein, albumin and fibrinogen polypeptide mRNA in different developing tissues. The findings suggest that complex mechanisms of gene regulation are involved in variable gene activities of α-fetoprotein, albumin and fibrinogen polypeptides in cells of different organs or tissues developed from a singla cell.

## Materials and Methods

*Materials.* All glassware and solutions were autoclaved prior to use. Ribonuclease-free sucrose, EDTA, phenol, sodium deoxycholate, salts and solvents were purchased from E. Merck; dithiothreitol and heparin (from porcine intestinal mucosa) were obtained from Sigma; deoxyribonucleoside triphosphates from Schwartz/Mann; Triton X-100 and SDS from BDH Chemicals Ltd. and [5-$^3$H]dCTP (spec. act. 18.4 Ci/mmol) was purchased from the Radiochemical Centre, Amersham. Oligo(dT)$_{12-18}$-cellulose (type T$_2$) and oligo(dT)$_{10}$ were from Collaborative Research, Inc., Waltham, MA. Avian myeloblastosis virus RNA-dependent DNA polymerase was kindly supplied by Dr. J.W. Beard, Life Sciences Inc., St. Petersburg, FL. S$_1$ nuclease (*Aspergillus oryzae*) was purchased form Miles Laboratories.

*Animals.* Sprague-Dawley rats were used throughout and were maintained on standard Purina Chow and water ad libitum. The embryonic and postnatal developing organs or tissues were isolated at different stages of development after killing the animals by decapitation under light ether anesthesia. No special attempt was made to remove the amnion for the isolation of yolk sac. Hepatomas were chemically induced in male Sprague-Dawley rats by feeding of 60 ppm diethylnitrosamine in drinking water for approx. 4 months [15]. All rats developed hepatomas producing α-fetoprotein within 4 months.

*Preparation of yolk sac polyribosomal RNA.* Yolk sac was obtained from rats at 15–16 and 19–20 days of gestation. After rinsing in ice-cold TMKMEDS buffer (20 mM Tris-HCl pH 7.4/5 mM $MgCl_2$/50 mM KCl/6 mM β-mercaptoethanol/0.1 mM EDTA/0.5 mM dithiothreitol/0.25 M sucrose), the polyribosomes were isolated according to the method of Miura et al. [7]. Polyribosomal RNA was prepared by addition of 0.5 vol. of extraction buffer containing 0.3 M NaCl/1.5% SDS/15 mM EDTA/30 mM Tris-HCl, pH 9.0. After incubation for 10 min at room temperature, several succesive extractions were performed with equal volumes of phenol/chloroform/isoamyl alcohol (50 : 48 : 2). When no detectable interface remained, 1/10 vol. of 3 M NaAc pH 5.2 and 2 vol. of cold ethanol were added. The RNA was precipitated overnight at −20°C and collected by centrifugation at 12000 × *g* for 20 min and resuspended in 10 mM Tris-HCl, pH 7.2, containing 0.5% SDS

*Preparation of polyribosomal RNA from developing organs or from hepatomas.* Isolation of polyribosomes from fetal and neonatal livers, kidneys, gastrointestinal tract and brain; from liver and kidneys of adult animals and from diethylnitrosamine-induced hepatomas was performed according to the method of Schimke et al. [16]. The polyribosomal RNA was prepared from the isolated polyribosomes as described earlier.

*Preparation of poly A containing RNA from polyribosomal RNA.* Polyadenylylated RNA was prepared by oligo(dT)-cellulose chromatography as reported [17].

*Preparation of purified mRNAs and $^3$H-labelled cDNAs.* Purification of mRNAs specific for α-fetoprotein, albumin and fibrinogen polypeptides, respectively, and the synthesis of the specific cDNA from these purified mRNAs have been reported previously [17–19].

*Analytical RNA-cDNA hybridization.* Analytical RNA-cDNA hybridization was performed according to the method of Housman et al. [20] in 5 μl sealed capillary tubes containing 0.2 M sodium

phosphate buffer (pH 6.8)/0.5% SDS/1000 cpm [$^3$H]cDNA (spec. act. $7.5 \cdot 10^6$ cpm/μg) and the required amount of RNA. The reaction mixture was heated at 100°C for 2 min and incubated at 65°C. After incubation, samples were diluted 400-fold into 30 mM sodium acetate, pH 4.5/300 mM NaCl/1 mM $ZnSO_4$/10 μg/ml denaturated and 5 μg/ml double-stranded calf thymus DNA. Hybrid formation was monitored by determining the percent of input [$^3$H]dCTP-labelled cDNA, which was insoluble in 10% trichloroacetic acid after digestion with 250 U/ml $S_1$ nuclease at 45°C for 30 min. Trichloroacetic acid-precipitable material was collected on nitrocellulose filter and counted by liquid scintillation spectroscopy.

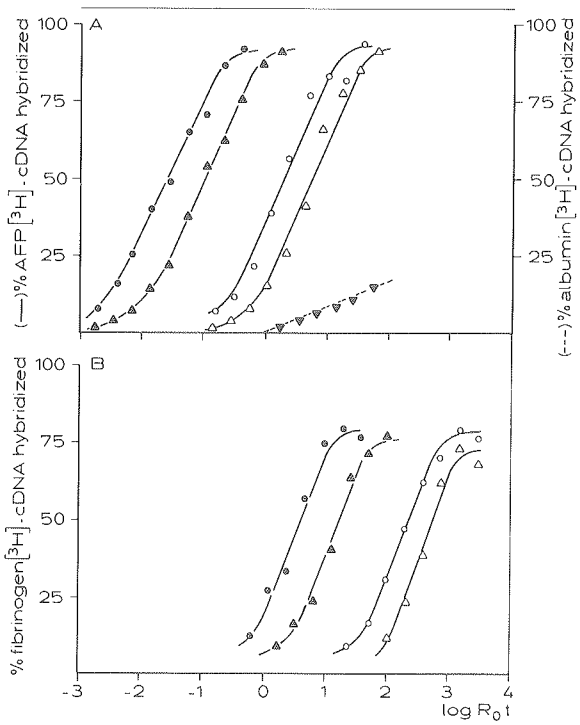


Fig. 1. Hybridization kinetics of rat yolk sac RNA isolated from 15 days and from 19 days of gestation. Total polysomal RNA (open symbols) and poly A containing RNA (closed symbols) prepared from yolk sac at 15 days (△,▲) and from 19 days (○,●) of gestation were hybridized to α-fetoprotein (A, solid lines), fibrinogen polypeptide (B) and to albumin, (A, dotted line) complementary DNA, respectively, using reaction conditions as described.

## Results

*Sequence content of α-fetoprotein mRNA, albumin mRNA and fibrinogen polypeptide mRNAs in rat yolk sac, liver, kidney, brain and gastrointestinal tract during fetal and neonatal development*

The concentrations of α-fetoprotein mRNA, albumin mRNA and fibrinogen polypeptide mRNA sequences were measured by molecular hybridization of the labelled specific cDNA probe to total polysomal RNA and to poly A containing RNA isolated from yolk sac, liver, kidney, brain, and intestines at different stages of fetal and neonatal development. As shown in Fig. 1, the concentration of α-fetoprotein mRNA and fibrinogen polypeptide mRNA sequences in RNA fraction extracted from yolk sac at different gestational age varies and increases during development. In contrast, albumin mRNA was not detected in total polysomal RNA isolated from the yolk sac.

The changes of α-fetoprotein, albumin and fibrinogen polypeptide mRNA concentrations in the neonatal developing kidney are illustrated in Fig. 2. The highest level of α-fetoprotein mRNA, albumin mRNA and fibrinogen polypeptide mRNA is found in the kidneys of one-day-old rats. In the polysomal RNA fraction isolated from rat kidneys 20 days after birth and from adult animals, these mRNAs cannot be detected by cDNA hybridization. There were also no detectable mRNA sequences for α-fetoprotein, albumin and fibrinogen polypeptide in the brain and gastrointestinal tract during fetal and neonatal life, even when hybridization reactions were carried out to significantly high $R_0t$ values (data not shown).

The α-fetoprotein, albumin and fibrinogen polypeptide mRNA levels in various RNA fractions isolated from rat liver at different stages of fetal and neonatal development are shown in Fig. 3.

The α-fetoprotein mRNA concentration in polysomal RNA fraction extracted from fetal and neonatal liver remained relatively constant up to 14 days after birth. In the 21-day neonatal liver, the level of α-fetoprotein mRNA was significantly lower, while in the 4-week-old and adult liver α-fetoprotein mRNA sequences were barely detectable (Fig. 3A). In contrast to α-fetoprotein mRNA, the albumin mRNA level in the liver

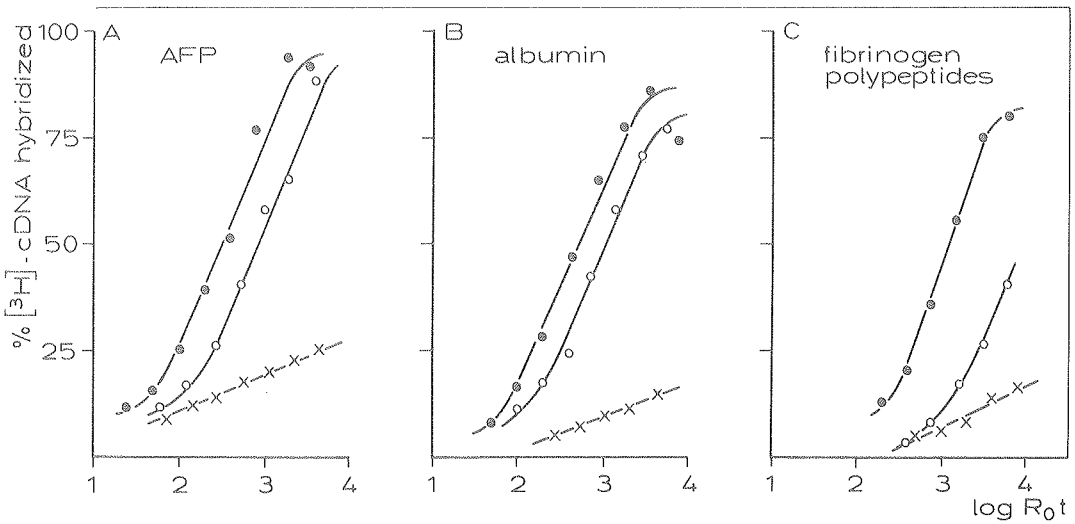


Fig. 2. Hybridization kinetics of rat kidney polysomal RNA prepared from rats, 1 day (●), 10 days (○) and 20 days (X) after birth. The polysomal RNA was hybridized to complementary DNA specific for α-fetoprotein (AFP) (A), albumin (B) and fibrinogen polypeptide (C), respectively, under reaction conditions as described.

increases steadily during fetal and neonatal development. As demonstrated in Fig. 3B, there is a 10-fold increase of albumin mRNA content throughout late gestation and the first 2 weeks of neonatal life. The changes of fibrinogen polypeptide mRNA content in the fetal and neonatal

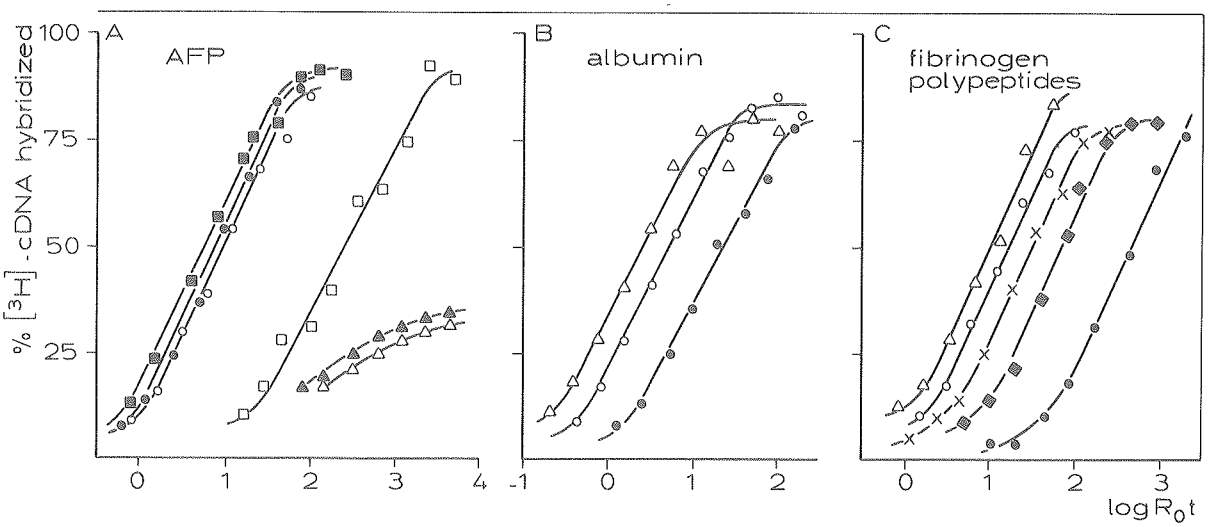


Fig. 3. Hybridization kinetics of polysomal RNA prepared from rat livers during fetal and neonatal development and from adult livers. The polysomal RNA was prepared from rat livers at 14 days (●), 17 days (◆), 21 days (X) of gestation, from livers of 1-week (○), 2-week (■), 3-weeks (□), 4-week-old (▲) rats and from adult animuls (△). The hybridization condition and specific cDNA probes used were as described in Fig. 2. The results of hybridization kinetics of liver RNA isolated from rats at 17 days and 21 days of gestation are identical with that of rats at 14 days of gestation for α-fetoprotein. For albumin, the findings from 17 and 21 days of gestation show no differences with that of 14 days of gestation. The results from 2-, 3- and 4-week-old rats are the same as those of the adult animals. For fibrinogen polypeptide, an identical result was found for liver RNA prepared from 2-, 3- and 4-week-old rats and from adult animals. AFP, α-fetoprotein.

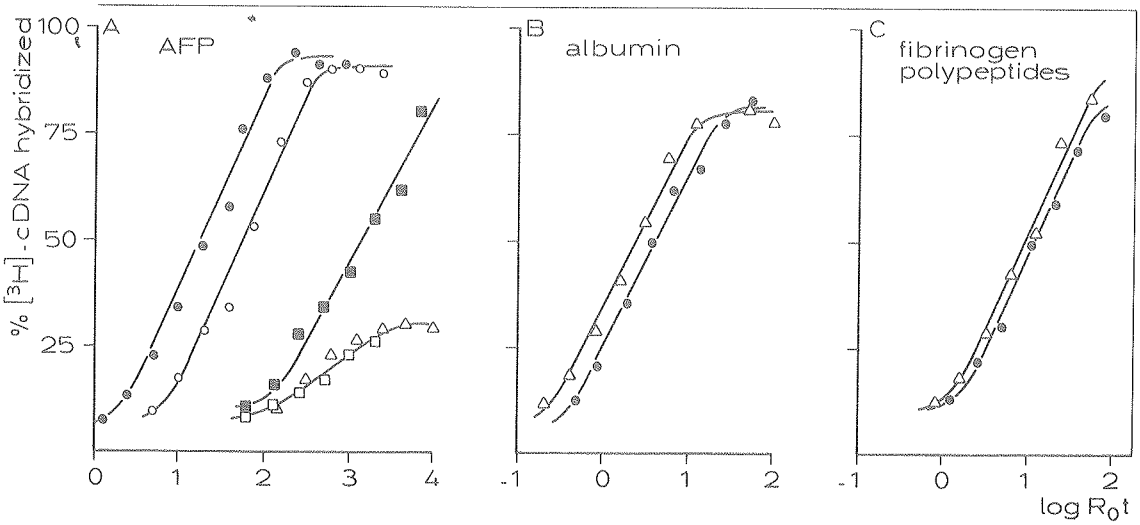


Fig. 4. Hybridization kinetics of polysomal RNA prepared from rat livers during diethylnitrosamine induced carcinogenesis. The polysomal RNA was prepared from livers of rats treated with diethylnitrosamine, as described under Materials and Methods, for 6 weeks (□), 10 weeks (■), 14 weeks (○), from liver tumor only (●) and from control normal rat livers (△). The hybridization reaction and specific cDNA probes used were as described in Fig. 2.

developing liver are shown in Fig. 3C. In 14-day-old fetal liver, there is a small quantity of fibrinogen polypeptide mRNA detectable ($R_0t_{1/2}$: 450 mol × s per l). However, there is a dramatic increase in mRNA content in the 17-day-old fetal liver (16-fold that of mRNA content of the 14-day-old fetal liver). In the liver of 2-week-old rats, the level of fibrinogen polypeptide mRNA is comparable to that found in adult liver ($R_0t_{1/2}$: 10 mol × s per l).

*α-Fetoprotein, albumin and fibrinogen polypeptide mRNA sequences in rat liver during carcinogenesis induced with diethylnitrosamine*

The changes in α-fetoprotein mRNA levels in adult livers of rats treated with diethylnitrosamine are illustrated in Fig. 4. By examining the $R_0t_{1/2}$ values of polyribosomal RNA isolated from rat livers at different periods after carcinogen exposure, from livers bearing hepatomas and from liver tumor only, we have found that the level of α-fetoprotein mRNA showed a steady increment during oncogenesis (Fig. 4A), while albumin and fibrinogen polypeptide mRNA levels remained constant (Fig. 4B, C). The level of α-fetoprotein mRNA in hepatoma is found to be as high as that in fetal liver.

## Discussion

Using immunological technique and the technique of incorporation of radioactively-labelled amino acids, Gitlin et al. [10] and Breborowicz and Nishi [11] reported that α-fetoprotein is produced by fetal liver, intestine, stomach and kidney. Using immunohistochemical techniques, α-fetoprotein has also been detected in the developing neuron [12]. In our present study, however, using molecular hybridization techniques, we were unable to demonstrate the presence of α-fetoprotein mRNA and albumin mRNA in fetal intestine and brain. The hybridization technique for detection of the site of synthesis of a specific protein is specific and sensitive [21]. The finding of AFP in fetal intestine and in developing neuron as reported (10–12) is, therefore, most likely due to cellular uptake of plasma protein.

The biological role of α-fetoprotein has been submitted to intensive investigation over the last few years. However, the specific function of this glycoprotein still remains unclear. α-Fetoprotein and albumin present some structural homologies [22], immunological cross reactivities and common chemical properties [23,24]. During gestation, the α-fetoprotein biosynthesis diminishes as albumin

synthesis and serum concentration rise. In the adult rat liver, phenotypic expression of α-fetoprotein is resumed during regeneration [4,25,26], during exposure to chemical hepatocarcinogens [15,25,27] and in hepatomas [3,4,15,28]. The mechanism leading to the recurrence of α-fetoprotein is still unknown. However, most of the processes which lead to α-fetoprotein reexpression cause albumin synthesis to diminish [29,30]. The coordinated reciprocal relationship of plasma levels of α-fetoprotein and albumin in ontogeny, as well as their similar physical and chemical properties [5,23,24], suggest that α-fetoprotein may be the 'fetal albumin'. The differential regulation of α-fetoprotein and albumin production represents therefore an important system for studying the control of gene expression. In the present study, we have examined the changes of the concentration of α-fetoprotein, and albumin mRNAs in rat liver, kidney, brain and intestine at different stages of gestation and postnatal period. The sequence contents of these mRNAs were also determined in the yolk sac during fetal development and in the liver during carcinogenesis induced with diethylnitrosamine. The results were compared with the findings of mRNA concentration of fibrinogen polypeptides as a marker of liver specific protein which is thought not to be related with the synthesis of α-fetoprotein. From this study, it is apparent that there is no consistent relationship between concentrations of albumin and α-fetoprotein mRNAs in different developing tissues. α-Fetoprotein, albumin and fibrinogen polypeptide mRNA sequences were all found in fetal and postnatal liver and kidneys, while in the yolk sac only α-fetoprotein and fibrinogen polypeptide mRNAs could be detected. In the liver, the increased level of albumin and fibrinogen polypeptide mRNA during fetal and neonatal development is accompanied by a diminished amount of α-fetoprotein mRNA. However, the neosynthesis of α-fetoprotein mRNA in the liver during carcinogenesis occurred without a decreased content of albumin and fibrinogen polypeptide mRNAs. These findings suggest that there is no reciprocal coupling mechanism of synthesis of albumin and of α-fetoprotein mRNAs. Although both albumin and fibrinogen are 'liver specific' proteins in adult life, the genetic expression of these two proteins in several developing organs also shows no consistent relationship. Moreover, intestines, which like the liver are also of endodermal origin, do not produce α-fetoprotein and albumin mRNAs, while kidneys which are of mesodermal origin were found to be α-fetoprotein, albumin and fibrinogen polypeptide mRNA producers in neonatal life. It is therefore clear that complex mechanisms of gene regulation are involved in variable gene activity in cells of different organs or tissues developed from a single cell. To investigate the control mechanisms of the synthesis of α-fetoprotein, albumin and fibrinogen polypeptide mRNA more directly, we have currently studied the resynthesis of α-fetoprotein and albumin mRNAs in a rat hepatoma cell line [18]. Evidence showed that α-fetoprotein and albumin mRNA levels in this cell line [18], but also in other cell lines (unpublished data) are dependent on species-specific serum in the growth medium. In contrast, the synthesis of fibrinogen polypeptide mRNAs is not influenced by the presence of species-specific serum. Further studies of the nature of this specific serum factor(s) and of the mechanism of action are in progress.

## Acknowledgements

This research was supported in part by a grant from KWF (Koningin Wilhelmina Fonds) and a grant from the Foundation for Fundamental Medical Research (FUNGO).

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