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No evidence for post-transcriptional control of albumin and α-fetoprotein gene expression in developing rat liver and neoplasia

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ABSTRACT

Rot analysis of hybridization data using highly labeled α-fetoprotein (AFP) and albumin (^{32}P)cDNA probes has been used to quantitate AFP and albumin mRNA sequences in RNA preparations from different subcellular fractions of developing rat liver and Morris hepatoma 7777. In addition, size analysis of these mRNA sequences has been carried out by electrophoretic fractionation on agarose gels containing methylmercury hydroxyde and hybridization to radioactive cloned albumin and AFP cDNA probes. In all the tissues examined (fetal, newborn and adult rat liver, and hepatoma 7777) most of the albumin and AFP mRNA sequences were found associated with the polysomes as mature mRNA molecules ; less than 2 % of these sequences were present in the nuclear or the non polysomal cytoplasmic compartments. The number of AFP mRNA molecules was found to decrease in parallel in all the cellular compartments during rat liver development. In Morris hepatoma 7777 the content of albumin mRNA was considerably decreased in all the cellular fractions as compared to normal liver. These results demonstrate that post-transcriptional control mechanisms leading to an accumulation of non-functional mRNA molecules are not implicated in the changes of expression of albumin and AFP genes during rat liver development and neoplasia.

INTRODUCTION

The serum proteins albumin and α-fetoprotein (AFP) provide a very good model system to investigate the molecular mechanisms responsible for changes in gene expression during developmental and oncogenic processes. These two proteins, synthesized by the mammalian liver, show a reciprocal relationship in their plasma levels (1, 2). AFP is the major serum protein of the developing fetus, where it is also synthesized by the yolk sac (3). AFP plasma levels decrease rapidly after birth to a residual level which is, in normal adult animals, 10^{-5} that of the fetal serum (4, 5). In rodents, this fall in the concentration of AFP in the neonatal serum results from a decrease of its rate of synthesis by the liver and the loss of the yolk sac. On the other hand, albumin is the major serum protein in adult animals and

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its concentration increases from low levels early in fetal development to a high constant level in post-natal life (2, 6). In the rat, albumin is not synthesized in significant amounts by the yolk sac (7, Sellem et al, in preparation). AFP synthesis by the adult liver is resumed under a variety of pathological conditions such as restitutive proliferation induced by partial hepatectomy or chemical liver injury, and exposure to chemical hepatocarcinogens (2, 8, 9). In addition, highly elevated levels of AFP reappear in the serum of adult animals bearing hepatomas or teratocarcinomas (10, 11). Most of the transplantable rat hepatomas which produce high levels of AFP show a reduced rate of albumin synthesis in relation to that of normal liver (12, 13).

Previous studies have shown that the production of albumin and AFP during rat liver development and in different transplantable hepatomas is regulated by modulating the steady-state concentration of the corresponding functional mRNAs (14, 15, 16). Furthermore, changes in albumin and AFP phenotypic expression in Morris hepatoma 7777 do not appear to involve modifications at the genome level, such as changes in gene number or gross rearrangements of the corresponding genes (17). The levels of functional mRNA molecules may then be regulated at the transcriptional level or be determined by post-transcriptional processes. Although transcriptional control mechanisms have been generally implicated in the expression of tissue specific proteins (18), recent experimental evidence has demonstrated post-transcriptional regulation of abundant mRNA species (19, 20).

To obtain further information on the control processes involved in the reciprocal expression of albumin and AFP genes we have carried out subcellular distribution studies of albumin and AFP mRNA sequences in rat liver at different stages of development and in Morris hepatoma 7777. The premise of this work was that changes in subcellular compartmentation of albumin and AFP mRNA sequences, during liver development and after neoplastic transformation, could be expected if changes in gene expression were under post-transcriptional control. In none of the cases examined we have found evidence for such changes ; most of the albumin and AFP mRNA sequences were invariably found associated with the polysomes as functional mRNA molecules.

MATERIALS AND METHODS

Materials and general procedures

The materials and general procedures used have been previously

reported (14, 21).

Preparation of whole cell RNA

Total RNA from adult liver and Morris hepatoma 7777 tissues was extracted by a modification of the phenol/m-cresol/SDS method of Liarakos et al. (22) as reported elsewhere (23). Total hepatic RNA from fetal and newborn (1 week) liver was isolated using the guanidinium thiocyanate procedure as described by Chirgwin et al. (24). Both methods yielded undegraded RNA preparations.

Preparation of nuclear and cytoplasmic RNA

Nuclei were prepared from rat liver and Morris hepatoma 7777 by a modification of the citric acid procedure of Bush (25) using 1 % Triton X-100. The supernatant resulting from the first 600 xg centrifugation step was promptly neutralized, made 1 % SDS and used to isolate the cytoplasmic RNA by phenol-chloroform extraction. Purified nuclei were lysed in 20 mM Tris-HCl buffer pH 8.0 containing 0.1 M NaCl, 2 mM EDTA and 1 % SDS ; nucleic acids were then extracted with an equal volume of phenol/ chloroform/isoamylalcohol mixture (49:49:2). Following ethanol precipitation RNA was separated from DNA by differential precipitation in 2.5 M LiCl at -4°C (26).

Preparation of polysomal and post-polysomal RNA

Polysomes were prepared from normal rat liver (fetal, newborn and adult) and Morris hepatoma 7777 as previously described (14). Polysomal RNA was isolated by a modification of the phenol/chloroform method of Perry et al (27) as reported elsewhere (21). Post-polysomal cytoplasmic RNA was extracted by the same procedure from the gradient fraction overlaying the 2.5/1 M sucrose polysome band.

Preparation of free and membrane-bound polysomes

Free and bound liver polysomes were obtained using the procedure of Ramsay and Steel (28) as modified by Yap et al (29).

Nucleic acid determinations

For chemical assays of RNA content in the different subcellular fractions nucleic acids were extracted essentially as described by Widnell and Tata (30), and RNA measured by the orcinol method of Ceriotti (31) with yeast RNA as standard. Final preparations of RNA were checked for DNA content by the diphenylamine method of Burton (32) using calf thymus DNA as standard. The contamination by DNA of total or nuclear RNA preparations was never found to exceed 1 % of the total nucleic acid content.

Synthesis of (^{32}P)-labeled DNAs complementary to AFP and albumin mRNAs

AFP and albumin cDNAs were synthesized from highly purified mRNA preparations (14) as reported elsewhere (23), using (^{32}P)dCTP (250 Ci/mmol) as labeled substrate. The (^{32}P)cDNA preparations were calculated to have a specific activity of $5x10^8$ cpm/µg.

mRNA/cDNA hybridization reactions

RNA excess hybridizations with albumin and AFP (^{32}P)cDNAs were carried out in sealed 10 µl or 100 µl siliconized capillaries as described previously (14, 23). Data were analyzed by using a computer program (33) designed to fit the data according to the equation :

$$c/C_o = P\,[1-\exp(-\ln 2 . Rot/Rot_{1/2})]$$

in which c/C_o represents the fraction of (^{32}P)cDNA in hybrid form at time, t, Rot = moles liter^{-1} sec. of nucleotides RNA, $Rot_{1/2}$ is the Rot value at 50 % hybridization, and P is the fraction of cDNA hybridized at the completion of the reaction.

Methylmercury hydroxyde-agarose gel electrophoresis of RNA

Size fractionation of RNA preparations on methylmercury hydroxyde agarose gels was carried out according to Chandler et al (34) with slight modifications : samples were made up in 10 mM methylmercury hydroxyde and vertical slab gels containing 1.2 % agarose and 10 mM methylmercury hydroxyde were used.

Transfer of RNA to cellulose paper and hybridizations to cloned albumin and AFP cDNA probes

After electrophoresis the RNA was transfered to diazobenzyloxymethyl cellulose (DBM)-paper according to Alwine et al (35), or to nitrocellulose paper by a modification of Thomas' procedure (36 ; Gal et al, in preparation). The RNA bound to the (DBM)- or to nitrocellulose paper was hybridized to (^{32}P)-labeled albumin or AFP recombinant cDNA probes, extensively washed and autoradiographed as described by Alwine et al (35).

The (^{32}P)-labeled cDNA probes used were prepared from two albumin cDNA containing plasmids (a 1:1 mixture of pRSA 13 and pRSA 57) or two AFP cDNA recombinant plasmids (a 1:1 mixture of pRAFP 65 and pRAFP 87), which include approximately 85 % of the corresponding specific mRNA sequence complexity (37,38). This was done by making use of the "nick translation" reaction catalyzed by E. coli DNA polymerase I essentially as described by Maniatis et al (39). The specific activity of the resulting recombinant (^{32}P)DNA was about 2-6 x 10^8 cpm/µg.

RESULTS

Highly specific albumin and AFP single-stranded cDNA probes (14) have been used to measure the concentrations of albumin and AFP mRNA sequences in total cellular RNA, nuclear, cytoplasmic, polysomal and post-polysomal RNA preparations isolated from rat liver at different stages of development and from Morris hepatoma 7777, which is a high AFP producer. The kinetics of hybridization of pure albumin and AFP mRNA, and of total and polysomal RNA from 19-day fetal rat liver to the ^{32}P-labeled cDNA probes are shown in Fig. 1 (A and B). The hybridization reactions are consistent with the cDNA probes reacting with a single species of RNA. The albumin ^{32}P-cDNA probe gave maximum hybridization values of about 95 % ; at completion, the AFP cDNA probe was protected to the extent of 80-85 %. $Rot_{1/2}$ values obtained with the pure mRNAs ($1.55x10^{-3}$ moles liter^{-1} sec. for albumin and $1.3x10^{-3}$ for AFP) are congruent with the sequence complexity of these mRNA species (14). As reported previously (17) under the stringent reaction conditions used in our experiments the albumin and AFP ^{32}P-cDNAs do not cross-react with the heterologous mRNA templates even at high Rot values.

Total RNA from fetal liver reacted more slowly with both probes than equivalent polysomal RNA preparations, indicating that polysomes are enriched in albumin and AFP mRNA sequences. The albumin and AFP $Rot_{1/2}$ values obtained with the total RNA are compatible with the data of Liao et al. (16) for polyadenylated RNA preparations from 19-day fetal liver, and indicate that albumin mRNA sequences are more abundant than AFP mRNA sequences at this stage of liver development.

Representative Rot curves corresponding to hybridization kinetic experiments of RNA preparations from different subcellular fractions of adult rat liver and Morris hepatoma 7777 are plotted in Fig. 2. Maximum hybridization values achieved for the albumin cDNA reactions with different RNA preparations from adult liver (Fig. 2A) and the hepatoma 7777 (Fig. 2C) were similar to those found with purified albumin mRNA from which the cDNA probe was transcribed (Fig. 1A). Similarly, the extent of hybridization of the AFP cDNA probe with the hepatoma 7777 preparations (Fig. 2D) was the same found for the back reaction to its template (Fig. 1B). In all cases, RNA isolated from the nuclear or post-polysomal fractions reacted 10 to 100-times more slowly than polysomal, cytoplasmic or total RNA preparations. This indicates that the concentration of albumin and AFP mRNA sequences in the nuclear or non-polysomal cytoplasmic compartments is much lower than in

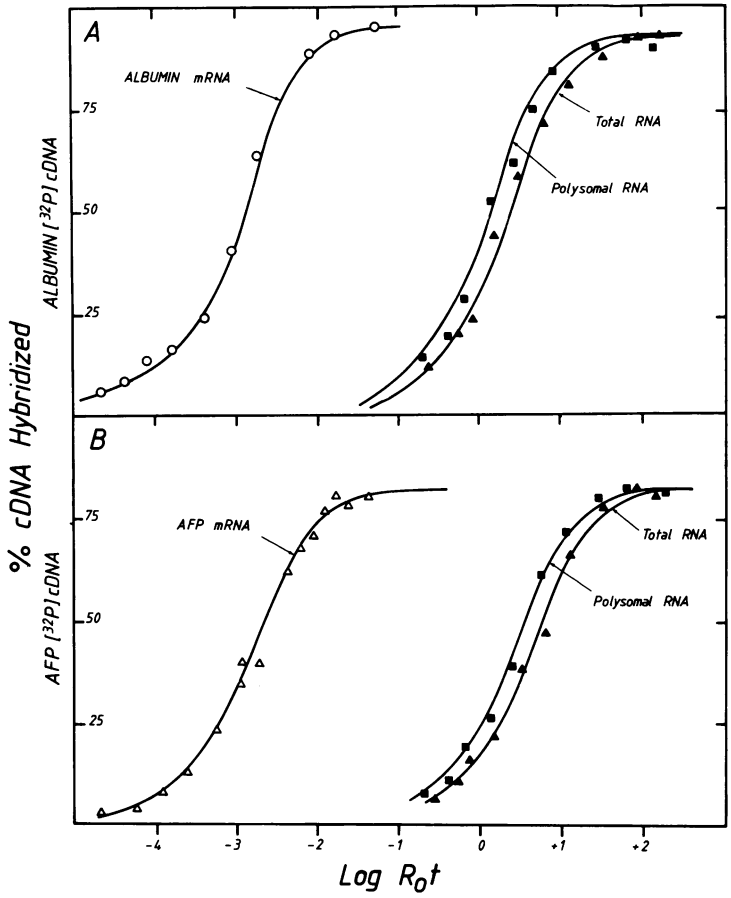

Figure 1 : Hybridization kinetic analysis of albumin and AFP mRNA sequences in total (▲—▲) and polysomal (■—■) RNA preparations from 19-day fetal liver. RNA excess hybridizations were carried out as described in Materials and Methods with [^{32}P]-labeled albumin (A) and AFP (B) cDNA probes. The Rot curves for pure albumin mRNA (○—○) and pure AFP mRNA (△—△) are also included. Computer analysis of the data provided the following $Rot_{1/2}$ values (in moles liter^{-1} sec.:A) Albumin cDNA : 1.5 x 10^{-3} for albumin mRNA ; 2.17 for total RNA ; 1.33 for polysomal RNA. B) AFP cDNA : 1.35 x 10^{-3} for AFP mRNA ; 4.03 for total RNA ; 2.56 for polysomal RNA.

the polysomes.

Total or polysomal RNA preparations from adult liver hybridized to the AFP cDNA probe at very high Rot values reaching maximum levels of about 70 % (Fig. 2B). These reactions carried out in final volumes of 10 µl did not go to completion probably for want of true RNA excess. Experiments

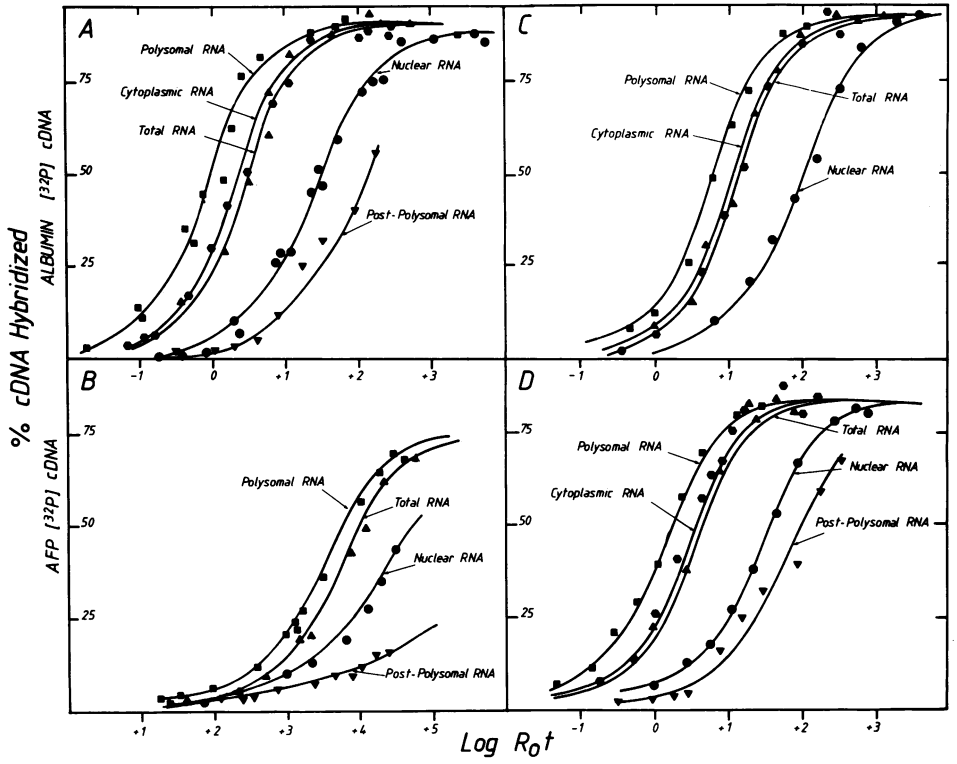

Figure 2 : Quantitation of albumin and AFP mRNA sequences in different subcellular RNA preparations from adult rat liver (A and B) and Morris hepatoma 7777 (C and D).

Total cell RNA (▲—▲), nuclear (●—●), cytoplasmic (⬣—⬣), polysomal (■—■) and post-polysomal (▼—▼) RNA preparations from adult rat liver and Morris hepatoma 7777 were hybridized to [^{32}P]-labeled albumin (A and C) and AFP (B and D) cDNA probes as described in Materials and Methods.

performed with high RNA concentrations and final reaction volumes of 100 µl gave maximum levels of hybridization of 85 % (not shown). Hybridizations observed under these conditions are highly specific since B. subtilis or yeast RNA failed to form significant amounts of hybrids at Rot values as high as $2x10^4$ moles liter^{-1} sec. The fidelity of the hybrids formed between the AFP cDNA probe and the total or polysomal RNA preparations from adult liver was determined by thermal denaturation experiments ; the stability of the hybrids was comparable to that observed for pure AFP mRNA-cDNA hybrids, a sharp melting curve with T_m of 89°C in 0.18 M NaCl (not shown). Although the Rot data shown in Fig. 2B do not allow to obtain very

precise quantitative values, they clearly indicate that, as found for the albumin cDNA reactions, nuclear and post-polysomal RNA react much more slowly than total or polysomal RNA preparations.

We have used data in Figs. 1 and 2 to calculate the mass fraction of RNA representing albumin and AFP mRNA sequences in the different subcellular RNA preparations. Using these values, the amount of RNA per cell and the molecular weight of the corresponding mRNA we have estimated the number of albumin and AFP mRNA molecules present in each cellular compartment. The results obtained for adult rat liver are compiled in Table I. In this table are also included $Rot_{1/2}$ values derived from hybridization reactions performed with membrane-bound and free polysomal RNA preparations, whose data are not presented in Figs. 2A and 2B to avoid overcrowding. There are approximately 21,000 molecules of albumin mRNA per adult liver cell, and most of these molecules (>88 %) are associated with the membrane-bound polysomal fraction. Only 6 % of the total mRNA molecules are found to be located on free polysomes which is similar to previously published values (29) ; less than 2 % are found in the nuclear or non polysomal cytoplasmic compartments. Although the number of AFP mRNA

TABLE I. SUB-CELLULAR DISTRIBUTION OF ALBUMIN AND AFP mRNA MOLECULES IN ADULT RAT LIVER.

RNA Fraction	$Rot_{1/2}$		Mass fraction of specific mRNA sequences in RNA of cellular compartment (a)		RNA content in cellular compartment in pg/cell (b)	Number of mRNA molecules per liver cell (c)	
	ALBUMIN	AFP	ALBUMIN	AFP		ALBUMIN	AFP
Purified mRNA	1.5×10^{-3}	1.35×10^{-3}	1	1			
Total RNA	2.05	$\sim 5 \times 10^{3}$	7.3×10^{-4}	2.7×10^{-7}	36.9	21,000	$\sim$ 8
Nuclear RNA	22.36	$>2.2 \times 10^{4}$	6.7×10^{-5}	$< 6 \times 10^{-8}$	1.8	96	<0.03
Cytoplasmic RNA	1.81	N.D.	8.3×10^{-4}	N.D.	33.2	21,600	N.D.
Total polysomal RNA	0.89	$\sim 3.5 \times 10^{3}$	1.68×10^{-3}	3.9×10^{-7}	16.2	21,380	$\sim$5
Bound polysomal RNA	0.82	$\sim 2.9 \times 10^{3}$	1.83×10^{-3}	4.6×10^{-7}	12.9	18,560	$\sim$5
Free polysomal RNA	2.75	$\sim 5.4 \times 10^{3}$	5.45×10^{-4}	2.5×10^{-7}	3.2	1,380	$\sim$0.6
Post-polysomal RNA	>90	$> 4 \times 10^{4}$	$<1.5 \times 10^{-5}$	$< 3 \times 10^{-8}$	11.0	<140	<0.2

N.D. Not determined
(a)(b)(c) Determined as indicated in Table II.

molecules in adult rat liver is very low (approximately 8 per liver cell), we have been able to determine that most of these molecules are located on bound polysomes.

Rot analysis has also been used to quantitate albumin and AFP mRNA molecules in newborn (1 week) rat liver. The data are presented in Table II (Rot curves are not shown). As found in adult liver, most of the albumin and AFP mRNA molecules are associated with the cytoplasmic polysomal fraction.

The calculation of the number of molecules of mRNA per cell in Tables I and II assumes that albumin and AFP mRNA sequences present in all the cellular compartments are mature mRNA molecules (M.W. 770,000 for albumin mRNA and 760,000 for AFP mRNA). To ascertain the nature of the albumin and AFP mRNA sequences detected in the Rot hybridization experiments the RNA preparations isolated from the different subcellular fractions were subjected to agarose gel electrophoresis in the presence of methylmercury hydroxyde. The RNA from the gel was transfered onto DBM-paper and hybridized to albumin or to AFP cDNA probes labeled with ^{32}P nucleotide triphosphates by nick translation. The autoradiographs of the "blot hybridizations" with the albumin (^{32}P)cDNA probe are shown in Fig. 3A. Both in total and polysomal RNA preparations from fetal (lanes 1 and 2),

TABLE II. CONCENTRATION OF ALBUMIN AND AFP mRNA SEQUENCES IN VARIOUS RNA PREPARATIONS FROM NEWBORN RAT LIVER (1 WEEK).

RNA Fraction	$Rot_{1/2}$		Mass fraction of specific mRNA sequences in RNA of cellular compartment (a)		RNA content in cellular compartment in pg/cell (b)	Number of mRNA molecules per liver cell (c)	
	ALBUMIN	AFP	ALBUMIN	AFP		ALBUMIN	AFP
Purified mRNA	$1.5x10^{-3}$	$1.35x10^{-3}$	1	1	-	-	-
Total RNA	2.21	30.7	$6.8x10^{-4}$	$4.4x10^{-5}$	36.9	19,550	1,283
Nuclear RNA	6.75	76	$2.2x10^{-4}$	$1.8x10^{-5}$	1.8	320	26
Polysomal RNA	0.98	13.39	$1.5x10^{-3}$	$1.0x10^{-4}$	16.2	19,410	1,380
Post-polysomal RNA	$>10^2$	$>10^3$	$<1.5x10^{-5}$	$<1.35x10^{-6}$	11.0	< 130	< 10

(a) Fraction of mRNA = Rot1/2 for pure mRNA hybridized to cDNA/Rot1/2 obtained for RNA fraction.
(b) Determined as described in Materials and Methods and taking into account the following : 1) the experimentally obtained RNA/DNA ratio in total liver tissue (= 4.2), and 2) a DNA content per rat liver cell of 8.7×10^{-12}g (40).
(c) Molecules of mRNA = Mass fraction (a) x RNA content (b) x 6.022×10^{23} molecules/molecular weight of specific mRNA. The molecular weights of albumin and AFP mRNA are taken to be 7.7×10^5 and $7.6x10^5$ respectively (14).

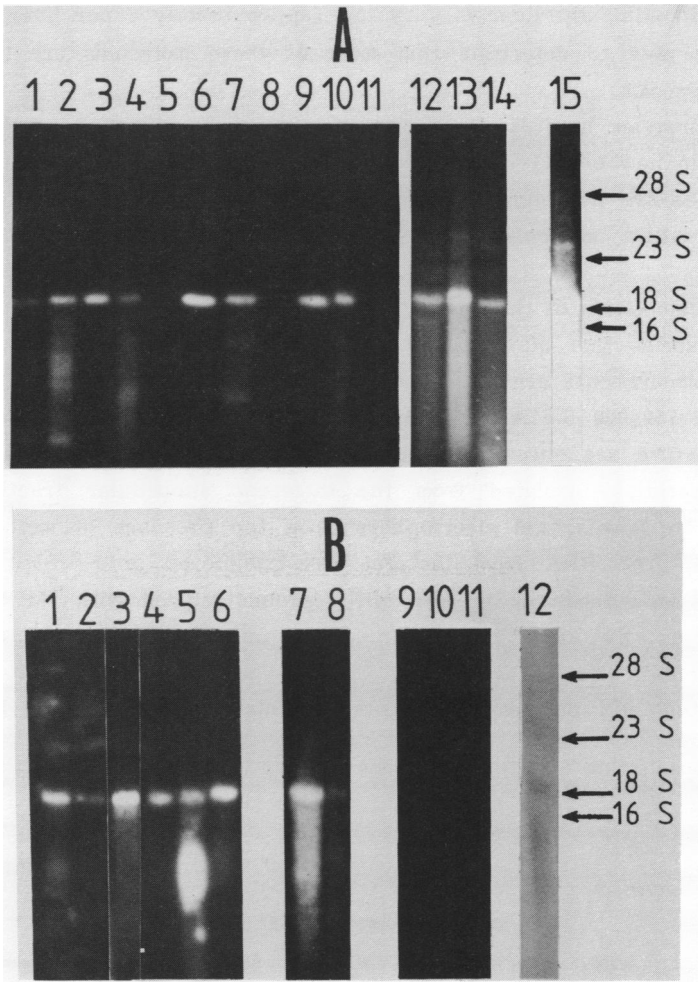

Figure 3 : Electrophoretic analysis of albumin (A) and AFP (B) mRNA sequences in different subcellular RNA preparations from developing rat liver and Morris hepatoma 7777.

RNA preparations from different subcellular fractions were separated in 1.2 % agarose slab gels containing 10 mM methyl mercury hydroxyde, transferred to DBM or to nitrocellulose paper (lane 12B) and hybridized with cloned albumin (A) or AFP (B) [^{32}P]cDNA probes as described in Material and Methods. The autoradiographs were exposed for 17 h with two intensifier screens except for the nuclear RNA sample in lane 16A which was overexposed for 43 h, and the nuclear and polyadenylated polysomal RNA samples in lanes 7B, 8B and 9B, and in lane 12B which were exposed for 24 h and 168 h respectively. 19-day fetal liver : Total RNA (lanes 1A, 1 µg, and 1B, 4 µg) ; Polysomal RNA (lanes 2A, 1 µg, and 2B, 0.5 µg). 1-week newborn liver : Total RNA (lanes 3A, 2 µg , and 3B, 9 µg) ; Nuclear RNA (lanes 14A, 9 µg, and 8B, 9 µg) ; Polysomal RNA

(lanes 4A, 1 µg, and 4B, 8 µg) ; Post-polysomal RNA (lane 5A, 30 µg). Adult liver : total RNA (lanes 6A, 2 µg, and 10B, 27 µg) ; Nuclear RNA (lanes 14A and 15 A, 14 µg and 9B, 14 µg) ; Polysomal RNA (lanes 7A, 1 µg, and 11B, 25µg) PolyA+ Polysomal RNA (lane 12B, 25 µg) ; Post-polysomal RNA (lane 8A, 30 µg). Morris Hepatoma 7777 : Total RNA (lanes 9A, 8 µg, and 5B, 2 µg) ; Nuclear RNA (lanes 13A, 22 µg, and 7B, 12 µg) ; Polysomal RNA (lanes 10A, 6 µg, and 6B, 1 µg) ; Post-Polysomal RNA (lane 11A, 30 µg). The arrows indicate the migration of 16 S and 23 S E. coli rRNA and 18 S and 28 S rat liver rRNA.

newborn (lanes 3 and 4) and adult (lanes 6 and 7) rat liver, and from the hepatoma 7777 (lanes 9 and 10) a discrete band can be observed which migrates in the position expected for a functional mature albumine mRNA molecule (2265 nucleotides ; 14). No hybridization signal can be seen with the different post-polysomal RNA preparations (lanes 5, 8 and 11) which is in keeping with the very low mass fraction values revealed in the Rot hybridization experiments.

In the nuclear RNA preparations (lanes 12, 13, 14 and 15) the bulk of the albumin mRNA sequences have a similar size to their polysomal counterparts. An intense band corresponding to mature mRNA is readily discerned. In addition, in all these preparations (newborn and adult liver, and hepatoma 7777) we observe a faint band corresponding to a larger RNA species (about 3700 nucleotides). This band which is more clearly visible in overexposed films (lane 15) does not appear to represent more than 5 % of the total albumin mRNA nuclear sequences. When hybridizing with the ^{32}P-labeled AFP cDNA probe, nuclear RNA preparations from newborn liver and hepatome 7777 revealed a single band (Fig. 3B, lanes 7 and 8) which run similarly to polysomal AFP mRNA (Fig. 3B, lanes 2, 4 and 6). The band observed in the polysomal and total RNA preparations from fetal and newborn liver, and the Morris hepatoma, corresponds to the size expected for mature AFP mRNA (2235 nucleotides ; 14).

Total, polysomal and nuclear RNA preparations from adult liver did not show any hybridization signal with the AFP cDNA probe (Fig. 3B, lanes 9, 10 and 11), as expected for the extremely low abundancy of AFP mRNA sequences in these preparations. However, using a polyA$^+$ fraction of adult liver polysomal RNA which was considerably enriched in mRNA sequences, and transfering the RNA to nitrocellulose paper by a modification of the Thomas' procedure (36) we were able to detect a band (lane 12B) corresponding to mature AFP mRNA. In addition there was a background of polydisperse components (<2200 nucleotides) which probably represent breakdown products of the mature AFP mRNA. Densitometric analysis of the

original autoradiographs shown in Figs. 3A and 3B gave results which were consistent with the relative albumin and AFP mRNA mass fraction values estimated by Rot analysis of hybridization in solution data.

The results of all these studies are summarized in Fig. 4 which shows the changes in the number of albumin and AFP mRNA molecules per cell in the different subcellular compartments during liver development and after neoplastic transformation of rat liver. The values given are not corrected for the fraction of liver cells represented by cell types other than hepatocytes. The total number of albumin mRNA molecules per liver cell gradually increases from around 16,000 in 19-day fetal liver to 22,000 in adult liver. In Morris hepatoma 7777 the concentration of albumin mRNA per cell is four

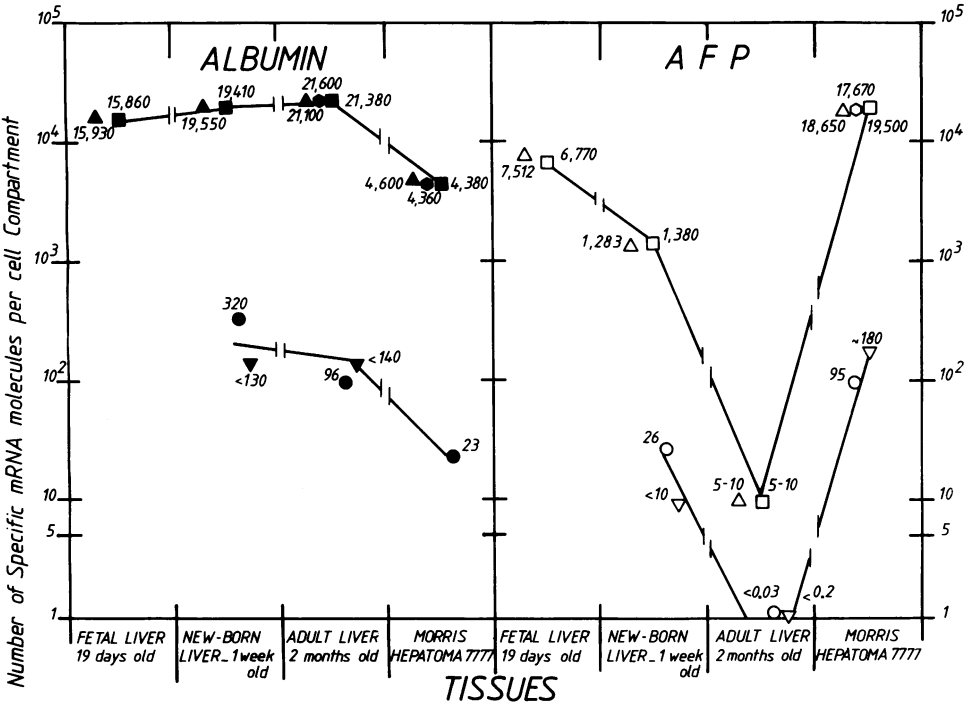

Figure 4 : Changes in concentration of albumin and AFP mRNA molecules in different cellular compartments during rat liver development and neoplasia.
Levels of albumin (black symbols) and AFP (open symbols) molecules per cell in the various cellular compartments at different stages of liver development and in Morris hepatoma 7777 were determined by Rot analysis of hybridization data as shown in Figs 1 and 2 and in Tables I and II. Whole cell (△═▲) ; Nucleus (○═●) ; Cytoplasm (⬡═⬢) ; Polysomes (□═■) ; Post-polysomal fraction (▽═▼).

to five-fold lower than in normal liver. The amount of AFP mRNA molecules per liver cell decreases exponentially from 7500 in fetal liver down to a 1000-fold lower level in adult liver. The concentration of AFP-specifying sequences in Morris hepatoma 7777 was at least 2000-fold higher than that found in normal adult liver. In all tissues examined the numbers of specific mRNA molecules in the polysomes almost fully account for the total cellular content, and are in agreement with published reports (13, 14, 16, 41, 42). In all stages of liver development and in Morris hepatoma 7777, we found very low levels of albumin and AFP mRNA molecules in the nucleus and in the post-polysomal cytoplasmic fraction ; these values are two orders of magnitude lower than their polysomal counterparts. Moreover, the concentration of specific mRNA molecules follows parallel variations in all the cellular compartments during rat liver development and in the hepatoma tissue. These results clearly show that gross changes in subcellular compartmentation of albumin and AFP mRNAs do not take place during liver development and after neoplastic transformation.

DISCUSSION

The final goal of our studies is to define the molecular mechanisms responsible for changes in expression of albumin and AFP genes during liver development and neoplasia. Previous work has indicated that the regulatory mechanisms are situated at a level prior to appearance of the corresponding mRNA molecules in the polysomes (13, 14, 15, 16). In this report we have attempted to develop evidence for an involvement of post-transcriptional processes in the regulation of polysomal albumin and AFP mRNA production. In particular, we have searched for an accumulation of non-functional AFP and albumin mRNA sequences in the nuclear and non polysomal cytoplasmic compartments of developing liver and Morris hepatoma 7777. Such a study was of special interest in states when the phenotypic expression of the proteins is considerably reduced (case of neonatal and adult liver for AFP, and of Morris hepatoma for albumin).

We have used a very sensitive hybridization technique with highly labeled albumin and AFP (^{32}P)-cDNA probes (specific activity 5×10^8 cpm/µg) which permited an accurate assessment of the number of molecules of the corresponding mRNA in the different subcellular compartments. This has allowed us to quantitate a steady-state level of 5 to 10 molecules of AFP mRNA per adult liver cell. In addition we have shown that most of these molecules are associated with the membrane-bound polysomal fraction and

have the size expected for mature AFP mRNA molecules. This strongly indicates that the few AFP mRNA molecules found in adult rat liver are functional mRNA molecules. This finding is consistent with the low residual levels of AFP observed in the plasma of normal adult animals (4, 5). These results may be due to a limited level of AFP expression by the whole adult hepatocyte population or, alternatively, they may be attributed to a considerable production of mRNA by a restricted number of developmentally arrested cells. At present it is not possible to exclude either of these possibilities. However, the results of our studies concerning the sensitivity of the albumin and AFP genes to nuclease digestion in liver nuclei (Nahon and Sala-Trepat, in preparation) would indicate that the AFP gene is transcriptionally active or at least has the potential for transcription in all adult hepatocytes.

Whatever cell population is involved in the production of AFP mRNA in adult liver our studies clearly show that there is no accumulation of AFP mRNA sequences in the nuclear or the non polysomal cytoplasmic compartments. In all tissues examined, only a very low, approximately constant proportion (about 1 %) of the total cellular content of albumin and AFP mRNA sequences was found in the nucleus. Moreover, the bulk of these nuclear sequences correspond to mature mRNA molecules. No RNA species containing AFP-specifying sequences larger than mature AFP mRNA molecules were detected in any of the nuclear RNA preparations analysed (neonatal liver and hepatoma 7777). In contrast, the existence of a high molecular weight species of RNA that contain albumin mRNA sequences was reproducibly observed in all preparations of liver and hepatoma nuclear RNA that were examined. This RNA species represents a small fraction of the total nuclear albumin mRNA sequences and its size (about 3700 nucleotides) is consistent with being a putative precursor to polysomal albumin mRNA. Evidence for the existence of a high molecular weight (26 S) presumptive precursor to albumin mRNA in the nucleus of rat liver has also been presented by Strair et al. (43). Since the albumin rat gene encompasses 15 kilobases of genomic DNA (37, 44), these RNA molecules could only represent late processing intermediates.

The steady-state distribution of albumin and AFP mRNA molecules in rat liver and hepatoma 7777 is very similar to that reported for other mRNAs specifying abundant tissue-specific proteins, like those coding for ovalbumin (45) and the immunoglobulins (46). It argues for an extremely efficient processing of the primary transcript and a rapid nuclear release of the

mature mRNA molecules to the polysomes.

We never found an accumulation of non-functional albumin and AFP mRNA sequences nor gross changes in the distribution of mRNA molecules in the different subcellular compartments. Accumulation of non-functional globin mRNA molecules in the nucleus has been observed in erythropoetic cells transformed by avian erythroblastosis virus (19) ; these cells fail to produce hemoglobin as a consequence of the post-transcriptional nuclear block. Post-transcriptional regulation of mRNA abundance shifts has been found associated with both developmental (20) and neoplastic (47) processes. Our studies provide no evidence for the operation of such mechanisms in the regulation of expression of the albumin and AFP genes in the system we are studying. They are rather consistent with transcriptional control of gene activity. However, the involvement of post-transcriptional control processes affecting the stability of the nuclear precursors or the cytoplasmic mRNA molecules can not be ruled out. To unambigously distinguish between transcriptional and post-transcriptional events in the regulation of albumin and AFP mRNA production, studies directed to determine the transcription rate of these genes in different tissues are in progress.

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