Changes in Rat $\alpha$-Fetoprotein and Albumin mRNA Levels during Fetal and Neonatal Development*

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Rat $\alpha$-fetoprotein (AFP) and albumin mRNA levels have been examined in yolk sac and liver during late gestation and early neonatal life by cell-free translation and RNA-excess cDNA hybridization. AFP-specific sequences were found to comprise up to 25% of the total poly(A)-containing RNA in the yolk sac, and they were reduced about 10-fold in the fetal liver. Since comparable amounts of poly(A)-containing RNA were obtained from both tissues, these observations suggest that the yolk sac may be the major source of maternal and fetal plasma AFP in late gestation. The level of AFP mRNA sequences in fetal liver remained relatively constant during the last week of gestation and the first 2 weeks of neonatal life. In contrast, the concentration of albumin sequences increased steadily during this time, reaching about 85% of adult levels by 2 weeks of age. These results suggest that the expression of AFP and albumin genes may not be reciprocally regulated, but rather they may be regulated independently of each other during the perinatal period. Albumin-specific sequences were also detected in the poly(A)-containing RNA from yolk sac but at a level of more than 400-fold lower concentration than that of the fetal liver.

It was especially noteworthy that the relative level of AFP sequences in the yolk sac decreased much earlier than that in the liver. Thus, AFP gene expression may be subject to different developmental controls in these two tissues. The continuation of AFP production in the liver following birth may indicate a continuing functional requirement for this protein in early neonatal life.

Rat $\alpha$-fetoprotein (AFP) and albumin are two major plasma proteins which show developmental regulation during fetal and neonatal growth (1, 2). AFP is a single chain glycoprotein ($M_r = 72,000$) containing about 4% carbohydrate (3, 4), whereas albumin is a single chain polypeptide with a molecular weight of approximately 66,000 and no prosthetic groups (5). In normal ontogeny, AFP is the dominant plasma protein throughout much of embryonic life. It is synthesized primarily by the embryonic liver and the yolk sac (1, 6, 7). Plasma AFP in rat embryos is first detected in mid-gestation and attains its highest concentration just prior to birth. It declines rapidly after birth until normal adult plasma levels of about 20 ng/ml are reached (8, 9). However, elevated plasma levels of AFP can be detected in adult animals during liver regeneration (1, 10) and in animals bearing hepatomas (1, 11). Albumin, on the other hand, is present at relatively low levels in fetal plasma, and its synthesis increases shortly before birth, then continues to increase toward adult levels (1, 7).

The coordinated reciprocal relationship of plasma levels of AFP and albumin in ontogeny, as well as their similar physical and chemical properties (2) and their amino acid sequence homology (12, 13), suggests that AFP may be the fetal analog of albumin. In addition, both proteins bind some of the same small molecules, including bilirubin (14, 15), copper (5, 16), and fatty acids (17, 18), which suggests a functional relationship between AFP and albumin.

The differential regulation of AFP and albumin production represents an important system for studying the control of gene expression of two related but different proteins under normal developmental conditions. In the present study, we have examined the alterations in the relative concentration of AFP and albumin mRNAs in the yolk sac and the liver during development. AFP and albumin mRNA levels were assessed by their translational activities in a cell-free protein synthesizing system and quantitated by analysis of the rate of hybridization between specific cDNAs and poly(A)-containing RNAs.

**EXPERIMENTAL PROCEDURES**

Rat serum albumin and rabbit anti-albumin were prepared as described (19). AFP was purified from the amniotic fluid of 19-day pregnant Sprague-Dawley rats by ammonium sulfate precipitation, affinity chromatography, gel filtration, ion exchange chromatography, and isoelectric focusing.‡ Purified AFP showed a single immunoprecipitation line with crossed immunoelectrophoresis against anti-amniotic fluid. Antibodies to purified AFP did not cross-react with serum albumin, and anti-albumin did not cross-react with AFP.

Yolk sacs or livers of Sprague-Dawley rats at different stages of development were collected, rapidly frozen in liquid nitrogen prior to homogenization, and extracted with phenol-chloroform as previously described (20). Poly(A)-containing RNAs were prepared by two successive affinity chromatography steps with poly(U)-Sepharose (21). Analysis of these RNAs by sucrose gradient sedimentation showed that essentially all ribosomal RNA species had been removed. Rat liver albumin mRNA and yolk sac AFP mRNA were purified by specific polysome immunoprecipitation and by size fractionation on isokinetic sucrose gradients, respectively (21-23).

Radioactively labeled cDNAs to albumin mRNA and AFP mRNA were prepared essentially as described (24), and AFP cDNA was further purified by the procedure of Alt et al. (25). Hybridization of cDNA with excess RNA was performed as previously described (26). Hybrids were measured by determining the S1 nuclease-resistant radioactive material. The results are expressed as the percentage of hybridization as a function of the log $R_s$. Hybridization data were consistent with pseudo-first order kinetics for RNA-excess cDNA hybridization reactions. The $R_s$ values for the hybridization reac-

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tions were obtained by using a computer program (27) to analyze the data to solve the equation $C/Co = 1 - \exp\left[-\frac{t}{Rd}\right]$ where $Co$ is the initial amount of single-stranded cDNA and $C$ represents the amount of cDNA in hybrids at time $t$ (28).

Liver and yolk sac hybridization data were compared to the $Rd$ values measured and calculated for albumin and AFP mRNA. A $Rd$ of $1.0 \times 10^{-5}$ mol·s·liter was determined for an albumin cDNA of 400 nucleotides in length and a $Rd$ of $1.32 \times 10^{-5}$ mol·s·liter was determined for an AFP cDNA of 280 nucleotides in length. Taking into account the cDNA lengths (29) and the hybridization conditions (salt concentrations), these values are similar to those reported by others (22, 30). The cDNA lengths were determined by sedimentation through alkaline isokinetic sucrose gradients (31). AFP and albumin mRNAs have been shown to have nearly identical lengths (about 2250 nucleotides) by gel electrophoresis under denaturing conditions. AFP cDNA:mRNA and albumin cDNA:mRNA showed sharp thermal transitions (data not shown) with $Tm$ values of 95° and 93°C, respectively.

Nuclei-treated rabbit reticulocyte lysate was prepared as described by Pelham and Jackson (32) with the modification described by Tse et al. (26). Total protein synthetic activity of the lysate reaction mixture was determined by examining the trichloroacetic acid-precipitable material. AFP and albumin translation products were measured immunologically with excess amounts of rabbit anti-AFP and anti-albumin, respectively, to ensure quantitative precipitation. The immunoprecipitates were purified through discontinuous sucrose gradients and the radioactivity was determined (19). Polyacrylamide gel electrophoresis of AFP and albumin immunoprecipitates showed that no other translation products contaminated these materials (data not shown).

RESULTS

The changes in AFP and albumin mRNA levels in the developing liver and the yolk sac were examined by their translational activities in the nuclei-treated reticulocyte lysate (Fig. 1). AFP synthesis varied in the yolk sac, and a maximum level occurred on Day 17 of gestation, where it constituted approximately 27% of total protein synthesis. Albumin synthesis, on the other hand, was not detectable in the yolk sac by direct immunoprecipitation. Both AFP and albumin synthesis could be measured in the liver at all ages studied with the exception of the adult liver, where AFP synthesis is too low for detection by this assay. Although the levels of AFP synthesis in the fetal liver were much lower than those in the yolk sac of the corresponding gestational age, they are higher than those of albumin synthesis. The relationship between AFP and albumin synthesis in the liver, however, was reversed on the last day of gestation, where albumin synthesis is greater than that of AFP. The difference in the levels of AFP and albumin synthesis reaches a maximum in the adult liver, where albumin synthesis consisted of about 12% of total protein synthesis and AFP synthesis fell to an undetectable level. The relationship of AFP to albumin production by the developing liver is shown as the ratio of AFP synthesis to albumin synthesis (Fig. 1, upper panel).

The concentration of AFP mRNA sequences was measured by hybridization of labeled AFP cDNA probe to poly(A)-containing RNA isolated from yolk sac and liver at different stages of fetal and neonatal development. Several representative hybridization curves are shown in Fig. 2, and the collected $Rd$ values, determined for all time points indicated, are presented in the legend to Fig. 2. By use of the $Rd$ values of the hybridization reactions (Fig. 2), the relative concentration of AFP mRNA sequences, as compared to total mRNA sequences, was estimated. Rates of hybridization between AFP cDNA and poly(A)-containing RNA samples from yolk sac varied with the gestational age, with the highest AFP mRNA level detected in the 17-day yolk sac. The rates of AFP hybridization remained relatively constant in the fetal

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**Fig. 1.** Changes in translational activities of AFP and albumin (Alb) mRNAs in yolk sac and liver during development. **Lower panel,** total $[^3]H$leucine-labeled translation products synthesized in reticulocyte lysates by mRNA from yolk sac or liver were incubated with antibodies to AFP or albumin. Immunoprecipitation of AFP from yolk sac ($O$) and liver ($\text{---}$) and of albumin from yolk sac ($\text{--}$) and liver ($\text{---}$) was performed as described (26). The extent of AFP and albumin synthesis was determined from their immunoprecipitable radioactivity and expressed as percentage of total trichloroacetic acid-precipitable counts. **Upper panel,** the developmental changes in the ratios of AFP and albumin synthesis as assayed by translation and immunoprecipitation.

**Fig. 2.** Quantitation of AFP mRNA sequences in rat yolk sac and liver during fetal and neonatal development. Total poly(A)-containing RNA of yolk sacs and livers from animals at different stages of development were hybridized in excess to purified AFP cDNA. Pooled yolk sacs, up to 12/pregnant rat, from at least 4 rats were collected for each time point and RNA samples were prepared from pooled livers of at least 20 fetal, 8 neonatal, or 4 adult rats. Several representative hybridization curves are shown. Total poly(A)-containing RNA from 15-day yolk sac, 45 ng (O); 19-day yolk sac, 45 ng (\text{--}); 15-day fetal liver, 90 ng (\text{---}); 19-day fetal liver, 90 ng (\text{---}); 1-day neonatal liver, 90 ng (O); 35-day neonatal liver, 900 ng (V), and adult liver, 5480 ng (\text{---}), was hybridized to 150 pg of AFP cDNA. The $Rd$ values (in moles·s·liter) for these data and for hybridization data not shown were: 7.59 × 10⁻⁵, 15-day yolk sac; 6.03 × 10⁻⁵, 17-day yolk sac; 6.31 × 10⁻⁵, 19-day yolk sac; 8.91 × 10⁻⁵, 21-day yolk sac; 5.25 × 10⁻⁵, 15-day fetal liver; 5.13 × 10⁻⁵, 17-day fetal liver; 5.01 × 10⁻⁵, 19-day fetal liver; 4.91 × 10⁻⁵, 21-day fetal liver; 7.31 × 10⁻⁵, 1-day neonatal liver; 6.01 × 10⁻⁵, 6-day neonatal liver; 5.94 × 10⁻⁵, 14-day neonatal liver; and 1.66 × 10⁹, adult liver.
liver and up to 14 days in the neonatal liver. However, the hybridization rates were significantly slower in 35-day neonatal liver and the adult liver, demonstrating 9- and 31,000-fold relative decrease, respectively, in AFP mRNA sequences from that of the 15-day fetal liver.

Labeled cDNA copies of the purified albumin mRNA were prepared (24) and used in RNA-excess cDNA hybridization reactions to determine the level of albumin mRNA sequences in the developing liver and yolk sac. Hybridization data are presented in Fig. 3. In contrast to AFP mRNA, albumin mRNA levels in the liver increased with the age of the animal, from about 1% of the total poly(A)-containing RNA in the 15-day fetal liver to about 12% in the adult liver. Our results have shown an age-dependent consistent increase in albumin mRNA levels in the liver. In contrast, the data of Sala-Trepat et al. (22) suggested that neonatal liver might contain somewhat more albumin mRNA than the adult liver.

The kinetics of hybridization was also employed to gain information regarding albumin gene expression in the yolk sac. When poly(A)-containing RNA from 19-day yolk sac was used in a RNA-excess cDNA hybridization with albumin cDNA, the rate of hybridization occurred about 450 times slower than that of the 19-day fetal liver (Fig. 3). From the observed Ra/2 values, albumin mRNA was calculated to comprise approximately 0.0054% of the total poly(A)-containing RNA from the 19-day yolk sac. To examine whether this low degree of albumin gene expression is specific for the yolk sac as a result of limited transcriptional activity, albumin cDNA was hybridized to total poly(A)-containing RNA prepared from rat kidney, spleen, and brain. As shown in Fig. 3, there were no detectable albumin mRNA sequences in these tissues even when hybridization reactions were carried to significantly higher Ra values.

The changes in AFP and albumin mRNA levels, determined by cDNA hybridization, in the yolk sac and liver at different phases of fetal and neonatal life are illustrated in Fig. 4. The results are similar to the data from the translation assays (Fig. 1) in that AFP mRNA sequences in the yolk sac are much higher than those of the corresponding liver. By examining the Ra/2 values, the level of AFP mRNA in the liver remained relatively constant throughout late gestation and the first 2 weeks of neonatal life, while albumin mRNA levels increased steadily and showed approximately a 9-fold increase during the same time period. Therefore, the age-dependent change in the ratio of AFP mRNA levels to albumin mRNA levels (Fig. 4, upper panel) reflects the consistent increase in albumin mRNA concentrations with AFP mRNA concentrations remaining relatively constant. These results suggest that the expression of AFP and albumin genes may not be reciprocally regulated, in contrast to the conclusion obtained from studies which examined only the plasma levels of AFP and albumin (1, 7, 33, 34).

**DISCUSSION**

Hybridization and cell-free translation studies have shown that the levels of AFP mRNA sequences are approximately 20% of the total poly(A)-containing RNA in the yolk sac. The levels of AFP mRNA in the fetal livers, however, were found to be about 5- to 10-fold lower than those in the yolk sac of corresponding gestational age. Since the yield of total poly(A)-containing RNA from the yolk sac (24 pg/g of tissue) is about the same as that of the fetal liver (28 pg/g of tissue), the yolk sac appears to be the major source of AFP in the amniotic fluid and fetal serum during rat fetal development. This conclusion is supported by the observation that there is a rapid postpartum fall in AFP serum concentrations (11) which is probably caused by the degradation or loss of AFP contributed by the yolk sac. Furthermore, yolk sac tissue placed in culture at 19-day gestation secretes approximately 4 times as much AFP as 19-day fetal liver tissue cultures (35).

AFP mRNA sequences decreased in the liver about 30,000-fold during the overall development of the liver. A low level of AFP mRNA sequences could be detected in the adult liver, consistent with the previously reported low basal level of plasma AFP (8, 9). This degree of AFP gene expression in the
adult liver may be due to a limited amount of transcription which remains from fetal gene activity. Alternatively, a continuous low level of AFP gene transcription may be due to ongoing regeneration of damaged hepatocytes that might arise during normal tissue maintenance.

It is especially noteworthy that, while the level of AFP mRNA sequences in the yolk sac began to decrease after Day 17 of gestation, AFP gene expression in the liver was maintained during the late gestational period and continued into early neonatal life. Thus, AFP gene expression may be subject to different developmental controls in these two tissues. The continuation of AFP production in the liver following birth may indicate a continuing functional requirement for this protein in early neonatal life. These changes in AFP mRNA levels in the yolk sac might reflect changing cell types in this tissue as parturition approaches. In the liver, however, previous studies have shown that AFP and albumin are synthesized in the same cell type (33).

In contrast to the high concentration of AFP mRNA in the yolk sac, albumin mRNA sequences are present at a very low level in this tissue. This observation may explain a previous inability to detect albumin protein by immunological techniques in the rat yolk sac (36, 37). In the case of the liver, albumin gene expression depends on the stage of liver development. For example, albumin mRNA sequences in the fetal liver consisted of only about 2% of the total poly(A)-containing mRNA, but its level increased to nearly 12% in the adult liver. This increase in albumin mRNA levels cannot be fully accounted for by an increase in the number of hepatocytes in the liver or by an increase in the number of hepatocytes that produce albumin, since the number of hepatocytes in the liver remains constant by 1 week after birth (38) and almost all hepatocytes synthesize albumin in the 21-day-old fetus (33). These observations suggest that the rise in albumin mRNA levels with liver maturation represents an increased albumin gene activity in the adult liver over that in the fetal liver.

The results presented here indicate that expression of AFP and albumin during development is most likely being regulated by the amount of corresponding functional mRNAs. It is especially noteworthy that AFP gene expression in the liver and yolk sac is under differential regulation and that AFP and albumin mRNA levels do not show direct reciprocal relationship during the perinatal period.

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