Homology between Rat Liver RNA Populations during Development, Regeneration, and Neoplasia

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ABSTRACT

To investigate the degree of homology which may exist between rat liver RNA populations during development, regeneration, and neoplasia, we hybridized polyadenylated RNAs from (a) normal adult, (b) 24-h regenerating, (c) 20-day fetal livers, and (d) the transplantable Morris hepatoma 5123tc to homologous and heterologous complementary DNAs and to cDNAs enriched for sequences preferentially transcribed in either adult or fetal liver. We also compared the in vitro translation products of these RNAs. Analyses of normal adult, regenerating, and fetal liver RNA populations and their translation products show that the overall pattern of gene expression during liver regeneration differs little from that of normal adult rat liver and that mature hepatocytes do not appear to revert to an "immature" state upon reentering the cell cycle. Comparisons between fetal, normal adult, and tumor RNA populations revealed that RNA populations from fetal liver and the 5123tc tumor lack sequences normally expressed in the mature adult liver. However, the tumor does not "reexpress" sequences which are preferentially expressed in fetal livers.

INTRODUCTION

During liver carcinogenesis and in hepatomas, the expression of fetal liver isozymes (1) and fetal antigens such as α-fetoprotein (2–4) frequently occurs. Based on these observations it has been suggested that most liver tumors result from the "dedifferentiation" or "retrodifferentiation" of mature hepatocytes (5). Similarly, the process of compensatory liver growth (liver regeneration) has been explained by some as encompassing hepatocyte retrodifferentiation and cell division followed by subsequent redifferentiation. The expression of fetal genes by neoplastic or neoplastic liver need not, however, imply the dedifferentiation of mature cells. Several lines of evidence have prompted the idea that neoplastic hepatocytes may arise from an immature liver cell type within an inconspicuous and as yet uncharacterized stem cell compartment (6, 7). Recently, we have shown that the proliferation of a relatively undifferentiated "oval cell" population is responsible for the reappearance of liver isozymes and α-fetoprotein mRNA during experimental hepatocarcinogenesis in rats fed a choline deficient diet containing 0.1% ethionine (8, 9). Furthermore we have found that during liver regeneration after carbon tetrachloride injury, full length α-fetoprotein mRNA molecules are synthesized primarily by nonparenchymal cells.4

Models which specify dedifferentiation as a necessary step in neoplastic and regenerative liver growth require that the overall pattern of gene expression in liver tumors and in regenerating liver reflect a less differentiated state than that of the adult liver. Although in several animal systems differentiation involves qualitative alterations in gene expression (10, 11), extensive characterizations of adult, regenerating, and neoplastic liver RNA populations have shown that such changes do not occur during liver regeneration (12–14) and hepatocarcinogenesis (15, 16). Obvious changes in gene expression which accompany regenerative liver growth and experimentally induced liver neoplasia are restricted to change in the abundance of certain liver RNA transcripts.

Our own data and those of Friedman et al. (17) on the abundance of albumin mRNA during liver regeneration indicate that differentiated characteristics of hepatocytes are not lost as the cells undergo replication. Friedman et al. and Powell et al. (17, 18) examined the expression of 11 cDNA α4 clones which are preferentially expressed in adult mouse liver and concluded that the expression of the mRNAs corresponding to these clones changes little during mouse liver regeneration, although they are variably expressed during development. These studies give information on sets of individual mRNAs and need to be complemented by an analysis of the overall homologies and differences existing between RNA populations during liver growth. For this purpose we compared RNA populations from 20-day fetal livers, from normal and regenerating adult livers, and from the Morris hepatoma 5123tc. We hybridized in liquid phase rat liver poly(A) RNAs to homologous and heterologous cDNA probes and to cDNA probes selectively enriched for RNA sequences preferentially transcribed in either adult or 20-day fetal livers. In addition we compared the in vitro translation products of poly(A) RNAs from fetal, adult, regenerating and neoplastic livers.

MATERIALS AND METHODS

Animals. Adult normal and regenerating livers were obtained from male Sprague-Dawley Holtzman rats (150–170 g). Partial hepatectomies were performed as described (13). Pregnant Sprague-Dawley Holtzman rats were obtained on day 18 of gestation and fetal livers were collected on day 20. A male Buffalo rat bearing the Morris hepatoma 5123tc was provided by Dr. W. E. Criss of Howard University and the tumor was maintained by serial i.m. transplantation in male Buffalo rats (100 g). Four weeks after transplantation recipient animals bearing palpable tumors were killed and the tumors were removed, dissected free of necrotic tissue, and frozen in liquid nitrogen. To deplete liver glycogen food was removed from adult rats 15–20 h prior to sacrifice (performed in all cases between 9 and 11 a.m.).

RNA Isolation. Frozen livers and tumor tissue were homogenized in a blender in 5 volumes of 75 mm NaCl:25 mm EDTA:0.5% SDS (pH 8.0) and 5 volumes of phenol. The homogenate was kept on ice for 30 min

4 The abbreviations used are: cDNA, complementary DNA; poly(A)*, polyadenylated RNA; MH 5123tc, Morris hepatoma 5123tc; RT, product of initial concentration of nucleotides in moles/liter multiplied by the incubation time in s; SDS, sodium dodecyl sulfate.
prior to centrifugation at 16,000 x g for 10 min at room temperature. The aqueous phase was stored on ice while both the organic phase and the interface were extracted with 2.5 volumes of phenol:chloroform (1:1, v/v) and 2.5 volumes of the blending solution. Aqueous phases were pooled, reextracted with an equal volume of phenol:chloroform, and then centrifuged as above. The resultant aqueous phase was treated with proteinase K (20 µg/ml) for 15 min at 37°C before one last reextraction with phenol:chloroform. Following ethanol precipitation total cellular RNA preparations were dissolved in 25 mM Tris (pH 7.0); 20 mM magnesium acetate; 25 mM NaCl; 2 mM CaCl₂ and were treated with isoosmolar-treated DNase (100 µg/ml) for 1 h at 37°C. Preparations were brought to 1% SDS, 5 mM EDTA, and 25 mM Tris (pH 7.4); extracted with chloroform:isoamyl alcohol (24:1, v/v); and centrifuged as above. The RNA was precipitated from the aqueous phase with ethanol. RNA precipitates were dissolved in 100 mM NaCl; 10 mM EDTA; 10 mM Tris (pH 7.5); 0.2% SDS and passed over a column of Sephadex G-100 equilibrated in the same buffer. Excluded fractions were collected and precipitated with ethanol. Poly(A)⁺ RNA was isolated on polyuridylic-Sepharose as described previously (13).

Nucleic Acid Hybridizations. Detailed methods for performing [³H]-cDNA syntheses, RNA-cDNA hybridizations and calculations have appeared in previous publications (13, 15). [³H]cDNA hybridization probes were transcribed from total cellular poly(A)⁺ RNA templates. RNA-cDNA hybridization reaction mixtures were made in 0.24–0.5 µM sodium phosphate (pH 6.9); 1 mM EDTA; 0.05% SDS and incubated at 68°C. The RNA concentrations used were 50 µg/ml and 1, 4, and 10 mg/ml, with RNA: cDNA ratios ranging from 1.25 × 10⁻¹ to 2.5 × 10⁻¹. The extent of cDNA hybridization was determined by digesting reaction mixtures with S₁ nuclease as previously described (13, 19).

"Enriched" cDNA Hybridization Probes. A cDNA probe enriched for sequences represented in adult liver RNA but absent or very rare in fetal liver RNA was obtained by annealing the [³H]cDNA transcribed from adult liver poly(A)⁺ RNA (0.125 µg; specific activity, 7 × 10⁶ cpm/µg) to an excess (150 µg) of 20-day fetal liver poly(A)⁺ RNA (0.24 µM sodium phosphate (pH 6.9); 1 mM EDTA; 0.05% SDS; 68°C; R₂ = 5 × 10⁵) and selecting the unhybridized cDNA fraction by hydroxyapatite chromatography as described (19). This fraction was then annealed to an excess (50 µg) of adult liver poly(A)⁺ RNA (R₂ = 5 × 10⁶) and treated with S₁ nuclease. RNA-cDNA hybrids were extracted with chloroform:isoamyl alcohol and treated with base (13, 19). Enriched adult liver cDNAs were chromatographed on Sephadex G-50 and the excluded fraction was precipitated with ethanol.

To obtain a cDNA probe enriched for sequences represented in fetal liver RNA but absent or very rare in adult liver RNA, it was necessary to select the unhybridized fetal liver cDNA fraction from two consecutive rounds of hybridization with excess adult liver RNA. [³H]cDNA transcribed from 20-day fetal liver poly(A)⁺ RNA (0.225 µg; specific activity, 6 × 10⁷ cpm/µg) was incubated with 225 µg of adult liver poly(A)⁺ RNA (R₂ = 5 × 10⁶) and the unhybridized cDNA fraction was incubated a second time with 25 µg of adult liver RNA to the same R₂ value. In each case hydroxyapatite chromatography was used to isolate the unhybridized cDNA fraction. Fetal liver cDNAs which failed to anneal with adult liver RNA were lastly incubated with 50 µg of 20-day fetal liver poly(A)⁺ RNA to a R₂ of 5 × 10⁶ and treated with S₁ nuclease. Enriched fetal liver cDNAs were purified from the RNA-cDNA hybrids as described above for enriched adult liver cDNA (see Chart 3).

In Vitro Translation. [³⁵S]Methionine-labeled translation products of liver poly(A)⁺ RNAs were prepared with a reticulocyte lysate system as described by the supplier (New England Nuclear) except that yeast RNA (50 µg/ml) was added. Translation products were denatured and separated by SDS:polyacrylamide gel electrophoresis (20, 21). Autoradiograms were scanned at 550 nm using a linear transport attachment and a Gilford 200 spectrophotometer. Molecular weight standards were from Bio-Rad.

RESULTS

Cellular Poly(A)⁺ RNA Populations in Adult and 20-Day Fetal Liver

The hybridization of adult and 20-day fetal liver poly(A)⁺ RNA with their homologous cDNAs is shown in Chart 1. Both reactions proceed over a broad range of R₂ values, an indication that adult and fetal liver poly(A)⁺ RNA populations are composed of sequences which are present in a wide but similar range of concentrations. Hybridization kinetics was analyzed using the computer program developed by Pearson et al. (22). The rationale for this type of analysis has been presented elsewhere (14). Briefly this program describes RNA populations as consisting of one or more frequency classes or components. Each class can be thought of as being composed of a variable number of different RNA species (referred to as the RNA sequence complexity), although by definition all members of any one class are represented at roughly similar concentrations within the RNA population (referred to as sequence abundance). RNA components are labeled class I, class II, etc., proceeding from the most abundant to the least abundant, respectively. Using this type of analysis our laboratory has shown previously that both adult liver polysomal and nuclear poly(A)⁺ RNA populations are best described using three components for each (19).
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Information from this type of kinetic analysis is clearly displayed by using derivative plots (23). Chart 1b is a derivative plot generated from the data presented in Chart 1a. A comparison of the two plots in Chart 1b shows that whole cell poly(A)+ RNAs obtained from either adult or 20-day fetal liver are each distributed in four frequency classes although the fraction of the total RNA mass within each component is different for each preparation (see also Table 1). Overall we estimate that adult and 20-day fetal liver total poly(A)+ RNA contain greater than 100,000 different sequences. Based on the hybridization rates (presented by $R_{d1/2}$ values) in Table 1, the first component of adult and 20-day fetal liver total poly(A)+ RNA is likely to be composed of relatively few, very abundant polysomal poly(A)+ RNA sequences. Much of this component in adult liver probably corresponds to liver specific RNA transcripts encoding albumin and other abundant serum proteins. Similarly, mRNAs encoding albumin and $\alpha$-fetoprotein are probably within the first component in 20-day fetal liver RNA. Compared to adult liver, class I RNAs constitute a much larger fraction of the total poly(A)+ RNA in the 20-day fetal liver.

A more detailed comparison of the data in Chart 1b and Table 1 indicates that the $R_{d1/2}$ value for component IV of fetal liver total poly(A)+ RNA is less than the corresponding value for adult liver RNA. This difference may indicate that (a) the rare RNAs [probably nuclear (19)] which constitute component IV in fetal liver hybridize at a faster rate because they are more abundant than the rare RNAs in adult liver or (b) that the total number of RNA sequences in component IV is greater in the adult liver. If true the latter alternative implies that in the rat a significant level of gene activation takes place during late fetal and neonatal liver development.

Homology between Adult and 20-Day Fetal Liver RNAs

Although the data above indicate that adult and 20-day fetal livers contain a similar number of RNA sequences, which are represented over a comparable range of frequencies (with the possible exception of some very rare RNAs), they give no clues as to whether or not the two hepatic RNA populations contain the same sequences. To examine this question a set of heterologous hybridization curves were generated. By comparing the saturation values for a set of homologous and heterologous hybridization curves, the extent of sequence overlap between two RNA populations can be determined. In addition careful analysis of the hybridization kinetics can provide information about changes in the abundance of sequences common to both RNA populations. The cDNA transcribed from adult liver poly(A)+ RNA was incubated with 20-day fetal liver poly(A)+ RNA and the cDNA transcribed from the 20-day fetal liver poly(A)+ RNA was incubated with adult liver poly(A)+ RNA. The resulting hybridiza-

Table 1

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<tr>
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<td>847.2</td>
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a Hydration reactions of total cellular poly(A)+ RNAs with cDNAs transcribed from total cellular poly(A)+ RNA preparations.

b Corrected $R_{d1/2}$, calculated $R_{d1/2}$ value of component class if that class were present alone in the RNA population.
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Chart 2. Hybridization of normal adult and 20-day fetal liver cDNAs with heterologous RNAs. a, heterologous hybridization of normal adult liver cDNA with the total poly(A)* RNA from 20-day fetal liver (C) and MH 5123c (B). —, homologous hybridization curve of adult liver cDNA with the total poly(A)* RNA from normal adult liver. b, heterologous hybridization of 20-day fetal liver cDNA with the total poly(A)* RNA from normal adult liver (A) and the MH 5123c (B). —, homologous hybridization curve of 20-day fetal liver cDNA with total poly(A)* RNA from 20-day fetal liver.

HAP Chromatography

nonhybridized cDNA hybridized cDNA (95%)

nonhybridized cDNA hybridized cDNA (8.1%)

total RNA + poly (A') total RNA from normal adult liver

Chart 3. Construction of "enriched" cDNA hybridization probes. Scheme for the selection of a cDNA probe to detect RNAs present in the normal adult liver but absent in the 20-day fetal liver (a) or RNAs present in the 20-day fetal liver but absent in the normal adult liver (b). See "Materials and Methods" for details of the selection protocol. HAP, hydroxyapatite.

20-Day Fetal Livers Contain Many, but not All, Adult Liver RNAs. The heterologous hybridization of adult liver cDNA with 20-day fetal liver RNA is extensive (Chart 2a). The ability of fetal liver RNA to hybridize with a majority of the adult liver cDNA is an indication that the polyadenylated RNAs of adult and 20-day fetal liver overlap considerably. The homologous and heterologous hybridization reactions obviously proceed with different kinetics; therefore the distribution of sequences within each class is not identical in the adult and fetal liver (Table 1). The kinetic data suggest that many adult liver RNAs are significantly underrepresented in the 20-day fetal liver.

As shown in Chart 2a and Table 1, the heterologous curve of adult liver cDNA with 20-day fetal liver RNA saturates at a level lower than the homologous hybridization curve of adult liver cDNA with adult liver RNA (80.0% versus 92.1%). Because 20-day fetal liver RNA is unable to form hybrids with the entire cDNA transcribed from adult liver RNA, it is very likely that 20-day fetal liver lacks some RNAs found in the adult liver. However, from these data alone one cannot discern whether 20-day fetal liver lacks only a few very abundant adult liver RNAs or up to several thousand much rarer sequences.

To investigate further the differences in adult and late fetal liver gene expression, we enriched our original adult liver cDNA probe for those sequences which are not represented in 20-day fetal liver RNA. This was done by subtractive hybridization as shown in Chart 3a and described in "Materials and Methods." When this "enriched" adult liver cDNA is incubated with homologous adult liver or heterologous fetal liver RNA, 74.2 and 37.6% of the cDNA form hybrids, respectively (Chart 4a). The sequences which are unique to the adult liver are those which hybridize at a faster rate and therefore correspond to the more abundant adult liver RNAs. The data suggest that qualitative gains in gene expression during later stages of rat liver development include the appearance of a relatively low number of abundant RNAs.

Adult Liver Lacks Some Fetal Liver RNAs. Because a majority of the RNAs found in the adult liver are also present in the 20-day fetal liver (Chart 2a) and the overall sequence complexity of the two RNA populations is roughly the same (Chart 1), it stands to reason that most of the RNAs found in the 20-day fetal liver should also be present in the adult liver. This reasoning proved to be correct when we compared the heterologous hybridization curve of 20-day fetal liver cDNA with adult liver RNA to the homologous hybridization of fetal liver cDNA with its template RNA (Chart 2b). The data points for the two curves overlap at Rf values greater than 2 x 10^4, which suggest that adult liver contains a majority if not all of the RNAs represented in 20-day fetal liver cDNA. However, computer generated saturation values (90.8% versus 85.4%) indicate that some fetal liver RNAs may be absent from adult liver. A more obvious difference
between adult and 20-day fetal liver RNA populations is made apparent by the striking dissimilarity of hybridization kinetics. When adult liver poly(A)$^+$ RNA is used to drive the hybridization of fetal liver cDNA, RNAs hybridizing as class I (abundant) sequences constitute a much smaller fraction of the total population than when fetal liver poly(A)$^+$ RNA is used (6.7% versus 30.1%). This marked difference in hybridization kinetics indicates that RNA sequences which constitute much of the abundant RNA class in 20-day fetal liver are produced at considerably lower levels, or may in fact be absent in the adult liver. These RNA sequences are likely to include globin and $\alpha$-fetoprotein mRNA among others. Although very abundant in fetal rat liver (24, 25), $\alpha$-fetoprotein mRNA represents only 0.006% of the adult liver polysomal poly(A)$^+$ RNA (26).

The data in Chart 2b and Table 2 suggest but do not conclusively demonstrate that certain abundant fetal liver RNAs are absent from the adult liver. To verify the existence of a set of RNA sequences which are present during late fetal liver development yet absent in the adult liver, we again used subtractive hybridization to isolate an enriched cDNA probe. Chart 3b illustrates the procedure used to enrich fetal cDNA for sequences not represented in adult liver RNA. The curve generated by the hybridization of fetal liver RNA with our "enriched" fetal cDNA probe. Chart 2a indicates that most of the RNAs found in adult liver. However, RNA from the hepatoma does not hybridize with all of the adult liver cDNA. Therefore the hepatoma must lack RNA sequences contained in the adult liver. Although fetal liver and hepatoma RNA hybridize with adult liver cDNA to similar extents (80.0% versus 79.1%), the two reactions proceeded with significantly different hybridization kinetics. The hybridization curves in Chart 2a indicate that some RNA sequences which are abundant in both the adult and 20-day fetal livers are present in lower concentrations in the MH 5123tc.

RNA from the hepatoma 5123tc hybridizes to less than 50% of the "enriched" adult liver cDNA (Chart 4a). Together with Chart 2a the data argue strongly that both fetal liver and the hepatoma each lack a set of sequences represented in the adult liver. Based on the similar hybridization kinetics of our "enriched" adult liver cDNA with either fetal liver or hepatoma RNAs, we suggest that the hepatoma RNA is deficient in adult liver RNAs which are also absent from 20-day fetal liver.

**Sequence Homology between Hepatoma and 20-Day Fetal Liver RNAs**

The extent to which gene expression in the hepatoma resembles that of 20-day fetal liver was examined by hybridizing 20-day fetal liver cDNA to hepatoma RNA (Chart 2b). This heterologous hybridization reaction saturated at a value slightly less than the corresponding homologous curve (84.2% versus 90.0% by computer analysis), which implies that the hepatoma is also deficient in RNAs contained in the 20-day fetal liver. A comparison of the curves in Chart 2b indicates that most of the RNAs which are abundant in the 20-day fetal liver are present in the hepatoma at even lower concentrations than in the adult liver. When "enriched" fetal liver cDNA is incubated with hepatoma RNA, only 46% is driven into hybrids (Chart 4b). The hybridization kinetics reveals that both the adult liver and hepatoma are deficient in RNAs which are abundantly represented within the "enriched" fetal cDNA probe. This finding suggests that the hepatoma may lack a set of late fetal liver RNAs which are also missing from normal adult liver and argues against the preferential expression of fetal liver genes in the tumor.

**Analysis of Regenerating Liver RNAs Using "Enriched" cDNA Probes**

Data presented in Chart 4 are consistent with our previous studies on gene expression during compensatory liver growth (14). These studies showed that there are few, if any, qualitative differences between RNA populations isolated from normal adult livers and from livers obtained at various times after partial hepatectomy. Unlike the fetal liver and hepatoma 5123tc RNAs, total poly(A)$^+$ RNA obtained from livers 24 h after partial hepatectomy is capable of hybridizing with a great majority, if not all, of the "enriched" adult cDNA (Chart 4a). Thus adult liver RNA sequences which are absent from the 20-day fetal liver or the hepatoma are maintained by the adult liver during regenerative

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growth. However, the rate at which this cDNA hybridizes with regenerating liver RNA suggests that the RNA sequences which are represented by this probe are less abundant in the regenerating liver.

**In Vitro Translation of Hepatic Poly(A)* RNAs**

The nucleic acid hybridization data indicate that a considerable degree of overlap exists between the polyadenylated RNA populations obtained from adult, 24-h regenerating, and 20-day fetal livers and the MH 5123tc. This finding is supported by the analysis of the *in vitro* translation products of the various hepatic RNA preparations. Figure 1 is an autoradiogram of[^35S]methionine-labeled translation products separated by SDS-polyacrylamide gel electrophoresis. The overall polypeptide profiles generated from the RNA of adult, 24-h regenerating, and 20-day fetal liver and the MH 5123tc are qualitatively very similar. Densitometric scanning of autoradiograms shows that this resemblance is particularly striking for the peptides synthesized from adult and 24-h regenerating liver mRNAs (Chart 5). Consistent with the findings of nucleic acid hybridization studies presented here and elsewhere (14), adult and 24-h regenerating liver RNAs can, however, be distinguished by quantitative differences in their *in vitro* translation products.

As expected fetal liver RNA generates a substantial amount of product which migrates at a position corresponding to globin. This prevents direct comparisons based on equivalent amounts of[^35S]methionine incorporation between fetal liver RNA translation products and those of the other RNA preparations. However, such comparisons can be made by using the albumin peak as an internal standard since albumin mRNA levels are equivalent in 20-day fetal and adult rat livers (27). Chart 5 shows that many, but not all, of the prominent polypeptides generated from adult liver RNA appear underrepresented when RNA from the 20-day fetal liver is translated. A doublet migrating at approximately M*, 70,000 in the fetal liver translation more than likely represents albumin and α-fetoprotein (double arrows). The larger of the two is the only major fetal product absent from the adult liver translation and thus probably corresponds to α-fetoprotein.

The *in vitro* translation of hepatoma RNA generates less M, 45,000–70,000 product than do either the adult or the regenerating liver RNAs. In particular very little material migrates at a position corresponding to albumin and/or α-fetoprotein (M, 70,000). This is consistent with our observation and with those
of Sell et al. (28) that the level of albumin mRNA in this hepatoma is 10-fold lower than in the normal adult liver and that α-fetoprotein mRNA represents only 0.014% of the polysomal poly(A)* RNA (data not shown). Conversely fetal liver RNA does not generate substantial amounts of a M, 38,000 translation product that is obvious in both the adult liver and hepatoma RNA translations (asterisk). These studies show that, although both the hepatoma and 20-day fetal liver are deficient in their ability to produce several adult liver mRNA transcripts, the adult mRNAs underexpressed in fetal and hepatoma RNA are not identical sequences.

**DISCUSSION**

A complete study of gene expression during normal and abnormal growth in the mammalian liver needs to include the analysis of the overall homology between RNA populations as well as measurements of the extent of expression of individual genes (17, 18, 29). In this paper we present a comparison at the protein mRNA represents only 0.014% of the polysomal poly(A)+ analysis of the overall homology between RNA populations as abnormal growth in the mammalian liver needs to include the establishment and maintenance of the cell phenotype. As described by Greengard (34) the latter stages of rat liver development are characterized by three bursts of newly expressed enzyme activities which occur at 18–20 days of gestation and at 3–4 and 20–21 days postnatally. Thus the appearance of ”new” RNA species during the latter stages of liver development indicated by our data is probably related to the acquisition of metabolic functions characteristic of the adult liver (34).

The inability of adult liver RNA to hybridize with a small fraction of the cDNA probe transcribed from 20-day fetal liver RNA suggested that certain RNAs present in the 20-day fetal liver may be absent from the adult organ. We confirmed this finding by enriching fetal liver cDNA for sequences not present in the adult organ and showing that a set of abundant fetal liver RNAs is not detectable in normal adult liver. This implies that hepatocyte maturation in the rat may involve genetic restriction as well as gene activation, an observation supported by the near absence of fetal liver isoforms and antigens in the adult liver and consistent with our recent finding that full length 2.3-kilobase α-fetoprotein mRNA found in fetal liver is absent from hepatocytes isolated from normal adult rat livers (39).

Fetal and adult liver poly(A)* RNAs also differ in the abundance of various sequences. Kinetic analysis of hybridization reactions indicates that in the adult liver some RNA sequences are present at higher levels than in fetal liver, while other sequences are more abundant in the liver at 20 days of gestation than in the adult. Comparisons of the in vitro translation product of 20-day fetal and adult liver RNAs support this finding even when corrected for the amount of globin translated from fetal liver RNA. Nevertheless we cannot completely exclude the possibility that additional sequences in the "enriched" fetal liver cDNA may correspond to RNAs preferentially transcribed in the hematopoietic cells present in livers at 20 days of fetal development (35). Thus changes in gene expression observed during late liver development may reflect maturational shifts in the cellular composition of the liver (i.e., the loss of hematopoietic cells), as well as a direct modulation of gene activity in the hepatocyte population.

Experiments utilizing "enriched" cDNA probes did not reveal qualitative differences between normal adult and regenerating liver RNAs and in vitro translation studies confirm that gene expression during regenerative growth deviates little from that of the normal adult liver. The data also show that sequences characteristic of adult liver and absent from 20-day fetal liver are retained by the adult liver during compensatory growth (24 h after partial hepatectomy) and that a set of RNAs preferentially expressed in the 20-day fetal liver is not reexpressed in regenerating livers. There are, however, shifts in the relative abundance of RNA sequences during liver regeneration as shown by the difference in the hybridization kinetics between normal adult and regenerating liver RNAs with an "enriched" adult liver cDNA probe. These observations are in complete agreement with earlier studies from this laboratory showing that extensive hepatocyte proliferation, characteristic of regenerative liver growth, can occur in the absence of major qualitative changes in hepatocyte gene activity (14). However, our analysis leaves out RNAs which are not polyadenylated. These species may show changes during liver growth processes as reported by Grady et al. (36).

Although primary tumors are certainly more useful in the study of neoplastic transformation in vivo (15), we used the transplantable hepatocellular carcinoma MH 5123tc in this study because it is a rapidly growing, relatively undifferentiated tumor. In contrast to gene expression in regenerating liver, gene expression in the MH 5123tc differs significantly from that of normal adult liver. Thirteen % of the hybridizable cDNA transcribed from normal adult liver poly(A)* RNA does not hybridize with the poly(A)* RNA isolated from the hepatoma, defining a set of adult liver RNAs absent from the tumor. These data are supported by the analysis of the translation products generated in vitro from adult liver and tumor poly(A)* RNA and are consistent with other reported studies (33, 37, 38). Because the tumor RNA also failed to hybridize appreciably with adult liver cDNA sequences which are not present in the 20-day fetal liver, adult liver RNA sequences which are lacking in the tumor, overlap to some extent to sequences which are also not detectable in the fetal liver. However, although the MH 5123tc is similar to the fetal liver in lacking some RNAs which are abundant in the adult, our data indicate that MH 5123tc does not contain a set of sequences which are preferentially expressed in 20-day fetal liver. Some of these results are not surprising given the undifferentiated nature of the tumor selected for study. Studies of individual RNA sequences and RNA populations need to be performed in relatively pure populations of transformed hepatocytes from primary tumors to obtain a more complete picture of the similarities and differences between the RNA populations of fetal and neoplastic livers.

In summary we show that the overall pattern of gene expres-
sion during liver regeneration deviates little from the normal adult pattern and gives no indication that mature hepatocytes revert to an "immature state" upon reentering the cell cycle. RNA populations from fetal liver and the 5123tc tumor lack sequences normally expressed in the mature adult liver. However, the tumor does not "reexpress" sequences which are preferentially expressed in fetal livers.

ACKNOWLEDGMENTS

We thank Anna-Louise Baxter for her help in preparing the manuscript.

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CANCER RESEARCH VOL. 45 OCTOBER 1985

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Homology between Rat Liver RNA Populations during Development, Regeneration, and Neoplasia


*Cancer Res* 1985;45:5114-5121.

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