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A Comparison of Polysomal Messenger Ribonucleoprotein Particles from Normal and Neoplastic Rat Liver[1]

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ABSTRACT

Free polysomes were isolated from normal and regenerating rat liver and from Morris hepatomas 7777, 7800, 5123C and 9618A. Sucrose gradient analysis ruled out the possibility of any significant messenger RNA degradation in these polysome preparations. The ethylenediaminetetraacetate-disrupted polysomes were fractionated on oligodeoxythymidylic acid-cellulose columns. The column-bound polyriboadenylic acid-containing messenger ribonucleoprotein particles were eluted with a formamide buffer, precipitated with ethanol, and subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. The messenger RNA-associated proteins from the different tissues were qualitatively similar, but two proteins with molecular weights of 66,000 and 109,000 found as minor proteins in normal liver appeared on gels as major protein bands when hepatoma messenger ribonucleoprotein particles were examined. The 66,000- and 109,000-molecular-weight proteins in these particles from regenerating liver appeared quantitatively similar to those isolated from normal liver.

INTRODUCTION

The heritable biochemical alterations sufficient to produce and maintain the neoplastic state remain unknown. The loss in control of some aspect of mRNA metabolism (transcription, transport, turnover, etc.) may play a critical role in carcinogenesis (10, 13, 14). mRNA exists in the form of a ribonucleoprotein particle, and the associated proteins may play a role in controlling its metabolism (3, 4–7). Methods to isolate these mRNA-associated proteins are now available (1, 3, 5–7). Since these proteins may include elements important in the regulation of translation, we decided to isolate mRNA-associated proteins from a number of rat hepatomas and to compare them to the proteins isolated from normal liver mRNP.

MATERIALS AND METHODS

Animals, Chemicals and Labeling. Male Holtzman rats (400 g) were used in the preparation of normal and regenerating liver mRNP.[3] Partial hepatectomies were performed 48 hr before the animals were sacrificed. Male Buffalo rats (200 g) bearing the tumors in their hind leg muscles were used to prepare hepatoma mRNP. The following hepatomas were examined: 7777 (4 weeks); 7800 (7 weeks); 5123C (9 weeks); 9618A (52 weeks). The numbers in parentheses refer to the time of growth in the host between transplantation of the tumors and sacrifice of the rats. Control experiments showed no qualitative or quantitative difference in the liver mRNP proteins from normal or regenerating Holtzman or Buffalo rats. Animals were given food and water *ad libitum*. The following buffers were used: Buffer A, 50 mM Tris-HCl (pH 7.5), 25 mM KCl, and 5 mM $MgCl_2$; Buffer B, 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, and 10 mM EDTA; Buffer C, 62 mM Tris-HCl (pH 6.8), 5% 2-mercaptoethanol, 10% glycerol, and 3% SDS.

Sucrose (RNase-free) and CsCl was purchased from Schwarz/Mann, Orangeburg, N. Y.; [^{3}H]orotate (21 mCi/mmol) was from Amersham/Searle Corp., Arlington Heights, Ill.

Polysomal mRNA Preparation. Free polysomes were isolated from normal liver and hepatomas as previously described (3). mRNA was specifically labeled in some experiments according to a protocol involving fluoroorotic acid and [^{3}H]orotate (2). Poly(A)-containing mRNP were separated from EDTA-dissociated ribosomal subunits with the use of oligo(dT)-cellulose columns (3).

Gradient Analysis. Ten A_{260} units of polysomes resuspended in Buffer A were layered onto 10 to 40% sucrose gradients made in Buffer A. Centrifugation was for 50 min at 40,000 rpm in an SW 41 rotor at 4°. Preformed CsCl gradients (1.3 to 1.75 g/ml) were centrifuged for 20 hr at 50,000 rpm in an SW 56 rotor at 20° after the application of 5 to 10 A_{260} units of polysomes in Buffer B. Fractionation and analysis of gradients were performed as described previously (3).

Gel Electrophoresis. Isolated mRNP were precipitated with 95% ethanol, washed, and dissolved in 0.4 ml of Buffer C. After 20 min of heating at 65°, 25 to 100 μl were layered onto SDS-polyacrylamide stacking gels and subjected to electrophoresis as described (3). After Coomassie Brilliant Blue staining, the gels were scanned at 550 nm in a Gilford 2400 spectrophotometer.

RESULTS

Prior to the isolation of the poly(A)-containing mRNP, we examined the polysome preparations to determine whether any mRNA degradation had occurred during their isolation. Polysomes from normal liver and the Morris 7777, 7800, and 9618A hepatomas were resuspended in Buffer A, applied to linear 10 to 40% sucrose gradients, centrifuged,

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[3] The abbreviations used are: mRNP, messenger ribonucleoprotein particles; SDS, sodium dodecyl sulfate; poly(A), polyriboadenylic acid; oligo(dT), oligodeoxythyidylic acid; p52, p66, p73, and p109, proteins with molecular weights of 52,000, 66,000, 73,000, and 109,000, respectively.

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and fractionated. Chart 1 shows that polysomes from all these tissues appeared relatively large (8 to 12 ribosomes per mRNA) with the presence of only a small amount of monosomes. Polysomes from the 7777 hepatoma, however, did appear to contain significant amounts of disomes. We conclude from this experiment that the isolated polysomes from the various tissues contain undegraded mRNP.

Poly(A)-containing mRNP were isolated on oligo(dT)-cellulose columns. Polysomes were disrupted with EDTA in the presence of 250 mM NaCl and applied to an oligo(dT)-cellulose column. The column was washed with application buffer, and the mRNP were then eluted with application buffer containing 25% formamide. Previous work has shown that by using this technique one can recover the majority of poly(A)-containing mRNP uncontaminated with ribosomal subunits (3). The eluate was precipitated overnight with ethanol, washed, and dissolved in Buffer C, and aliquots were subjected to electrophoresis on 8.75% acrylamide gels. Chart 2 shows the profile of gels scanned at 550 nm after being stained with Coomassie blue dye. The profile for the mRNP proteins from normal liver shows the presence of a number of proteins. Two of the prominent bands correspond to proteins with molecular weights of 52,000 and 73,000. The *arrows* point to minor proteins from normal liver mRNP with molecular weights of 66,000 and 109,000, designated p66 and p109, respectively. In the profiles for the mRNP proteins from the Morris 7777, 7800, and 5123C hepatomas, these minor proteins, p66 and p109, now appear to be major bands. The other hepatoma mRNP proteins appear qualitatively and quantitatively similar to the mRNP proteins from normal liver. The only exception is the presence of a protein with a molecular weight of 90,000

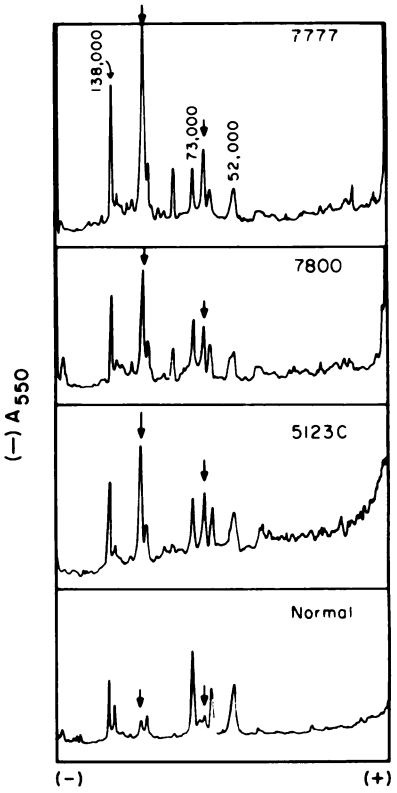

Chart 2. Gel electrophoresis of polysomal mRNP proteins. Polysomal mRNP were isolated from EDTA-dissociated free polysomes as described in "Materials and Methods." The ethanol-precipitated material was dissolved in Buffer C, and aliquots were subjected to electrophoresis on 8.75% polyacrylamide gels in the presence of sodium dodecyl sulfate.

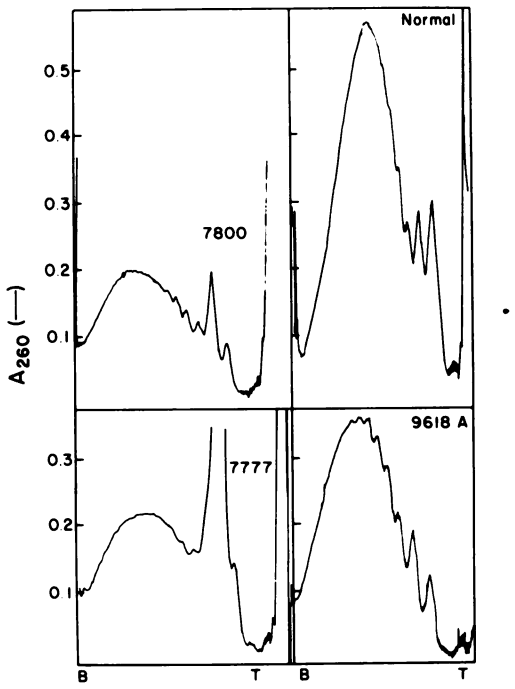

Chart 1. Sucrose gradient analysis of free polysomes. Free polysomes were isolated from normal liver and various Morris hepatomas as described in "Materials and Methods." The pellets were resuspended in Buffer A, and 10 A_{260} units were layered and centrifuged on 15 to 30% sucrose gradients as described in "Materials and Methods."

present in the 7777 and 7800 hepatomas that appears to be absent from normal liver and the 5123C hepatoma. The mRNP proteins examined thus far have been isolated from relatively fast-growing tumors. To determine whether the increase in the p66 and p109 proteins relative to the other mRNP proteins is related to rapid growth, we performed the following experiment.

Polysomal mRNP were isolated from a slow-growing tumor, 9618A, and from regenerating liver and subjected to electrophoresis. The protein profiles shown in Chart 3 reveal that mRNP from the slow-growing hepatoma, 9618A, contain large amounts of p66 and p109 relative to the other proteins present (compare to 7777 mRNP proteins). Furthermore mRNP proteins from regenerating liver appear quantitatively and qualitatively identical with those from normal liver. We quantitated the amounts of protein present on the gels by measuring the area under the peaks with a planimeter. The results are shown in Table 1. We have expressed the results as the ratio of the amount of p109 and/or p66 to the amount of p52 and/or p73. p52 and p73 were chosen as standards because the relative amount of these 2 proteins varied little from gels run on mRNP isolated from all the tissues used. The ratios of p66 or p109 to p52 or p73 were consistently elevated in the tumor mRNP when compared to similar ratios calculated for normal liver mRNP. Table 1, Column 6, shows the ratios calculated for the sum of p66 and p109 divided by the sum of p73 and p52. Table 1, Column 7, shows the increase in this calcu-

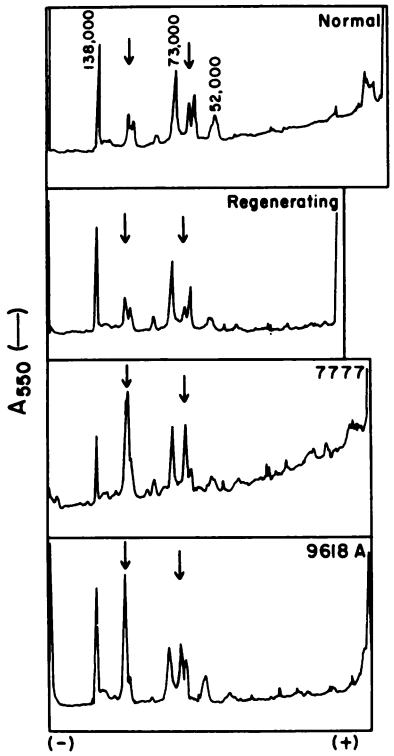

Chart 3. Gel electrophoresis of polysomal mRNP proteins. Polysomal mRNP were isolated and subjected to electrophoresis as described in Chart 2.

lated ratio for tumor mRNP protein bands relative to that calculated for normal mRNP proteins. The fastest growing hepatoma used in these experiments, 7777, shows the largest increase in this ratio. The increase in this ratio for the 3 other hepatomas ranges from 5.2 to 5.7.

We performed the following experiment to see whether the amount of protein per unit mRNA was greater in the hepatoma tissue than in normal liver. mRNA-labeled polysomes (see "Materials and Methods") were resuspended in Buffer B, fixed with formaldehyde, and subjected to CsCl gradient analysis (Chart 4). Labeled mRNP from normal liver bands at a density of about 1.47 g/ml and is well separated from the ribosomal subunits, which band at about 1.60 g/ml. The labeled mRNP from the 5123C and 7800 hepatomas show radioactive peaks in the area of 1.47 g/ml, which suggests that the RNA/protein ratios for the tumor mRNP are similar to those for normal liver mRNP. This suggests that the increased amount of p66 and p109 may be compensated for by a decrease in the other mRNP proteins or that these 2 proteins may bind a subset of mRNA, poorly labeled under our conditions. The radioactivity coincident with the A_{260} peak in the CsCl gradients of the tumor polysomes represents rRNA label. Our method for specifically labeling mRNA in normal liver is not as specific in these hepatomas as in liver, thus accounting for the labeling of some rRNA (results not shown).

Table 1
Quantitation of mRNP proteins

Tissue[a]	p109/p73	p109/p52	p66/p73	p66/p52	(p109 + p66)/ (p52 + p73)	Fold[b] increase
7777	3.02 ± 0.31	4.00 ± 0.05	1.40 ± 0.23	1.85 ± 0.10	2.51 ± 0.20	8.4
7800	1.83 ± 0.42	2.54 ± 0.59	0.86 ± 0.10	1.19 ± 0.12	1.56 ± 0.28	5.2
5123C	2.16 ± 0.48	1.18 ± 0.06	1.18 ± 0.08	1.35 ± 0.34	1.78 ± 0.42	5.9
9618A	1.76 ± 0.36	3.36 ± 0.28	0.86 ± 0.14	1.64 ± 0.12	1.71 ± 0.35	5.7
Normal	0.30 ± 0.08	0.30 ± 0.09	0.28 ± 0.11	0.32 ± 0.12	0.30 ± 0.07	1.0

[a] Hepatoma lines listed in order of decreasing growth rate.
[b] Determined by dividing value in Column 6 by that found for normal.

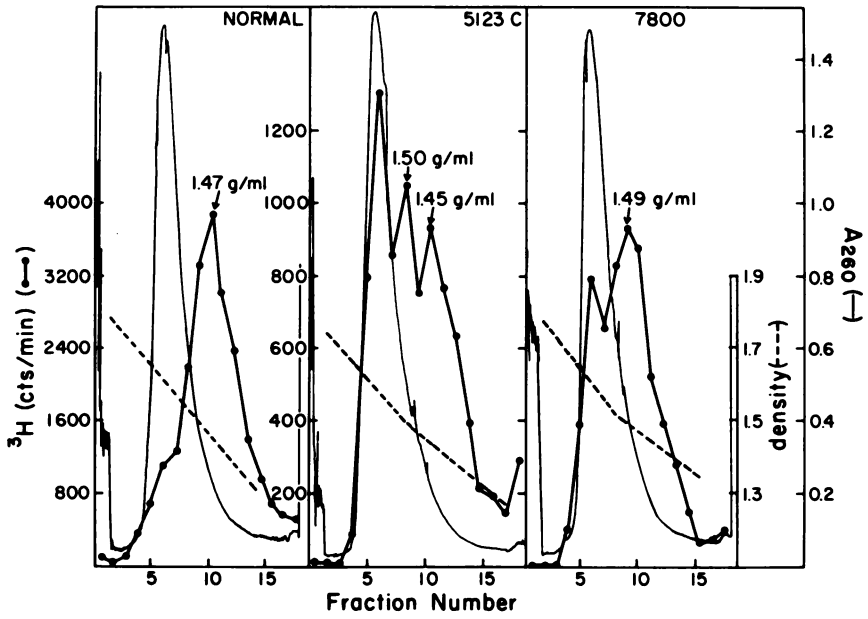

Chart 4. CsCl gradients of labeled polysomal mRNP. Isolated mRNA-labeled polysomes were resuspended in Buffer B. Aliquots were then layered and centrifuged on preformed CsCl gradients as described in "Materials and Methods." *cts*, counts.

DISCUSSION

Undegraded polysomal mRNP isolated from 4 different rat hepatomas appear quantitatively similar to normal liver mRNP in terms of RNA/protein ratios and qualitatively similar in the types of proteins present. We found, however, that the hepatoma mRNP contained 2 proteins seen as major bands on SDS-polyacrylamide gels that were found as minor bands on gels of normal liver mRNP proteins. The 2 proteins, p66 and p109, were also minor proteins in regenerating and host liver mRNP, suggesting that their increase in hepatomas was not due to tissue growth or to exposure to an altered hormonal environment *per se*. Furthermore these proteins were increased in the 9618A hepatoma which grows very slowly, a further indication that their increased occurrence in hepatoma mRNP was not related to growth rate. The possibility exists that the increase in certain mRNP proteins is related in some way to the initiation and/or maintenance of the neoplastic state. The relationship between the function of p66 and p109 and other mRNP proteins and the expression of neoplasia is unknown but might include the following.

mRNP proteins have been postulated to play a role in the selection and transport of mRNA sequences from the nucleus to the cytoplasm (12). The release of nuclear-restricted RNA to the cytoplasm has been suggested to be important in the process of carcinogenesis (13–15). Recent evidence has shown that the protein composition of liver nuclear ribonucleoprotein is altered by treatment of rats with carcinogenic aminoazo dyes (9).

The mRNA proteins may function in some step of protein synthesis, *i.e.,* initiation. The 5123C hepatoma contains more polysomal-associated initiation factors than normal or host liver does (8).

These proteins may be involved in determining mRNA half-life. mRNA half-life in a number of hepatomas appears different from the turnover of these species in normal liver (R. Moore and H. C. Pitot, unpublished data), and it has been suggested that the phenotypic heterogeneity observed for a variety of tumors may be related to this phenomenon (11). p109 and p66 are missing from membrane-bound mRNP in normal liver, and these proteins may play a role in the shift in polysomes from the membrane bound to the free class seen in hepatomas (3). Since endoplasmic reticulum membranes may affect mRNA stability (10, 11), this shift in the distribution of polysomes, perhaps influenced by p66 and p109, may alter the relative amounts of different mRNA's which is turn could affect levels of protein growth control factors.

Although this discussion is speculative, the questions posed are amenable to investigations that might determine whether alterations in the proteins involved in mRNA metabolism may alter control factors affecting growth and other properties peculiar to neoplastic cells.

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