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Comparison of Cytosolic and Nuclear Poly(A) Polymerases from Rat Liver and a Hepatoma: Structural and Immunological Properties and Response to NI-Type Protein Kinases†

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ABSTRACT: Poly(A) polymerases were purified from the cytosol fraction of rat liver and Morris hepatoma 3924A and compared to previously purified nuclear poly(A) polymerases. Chromatographic fractionation of the hepatoma cytosol on a DEAE-Sephadex column yielded approximately 5 times as much poly(A) polymerase as was obtained from fractionation of the liver cytosol. Hepatoma cytosol contained a single poly(A) polymerase species [48 kilodaltons (kDa)] which was indistinguishable from the hepatoma nuclear enzyme (48 kDa) on the basis of CNBr cleavage maps. Liver cytosol contained two poly(A) polymerase species (40 and 48 kDa). The CNBr cleavage patterns of these two enzymes were distinct from each other. However, the cleavage pattern of the 40-kDa enzyme was similar to that of the major liver nuclear poly(A) polymerase (36 kDa), and approximately three-fourths of the peptide fragments derived from the 48-kDa species were identical with those from the hepatoma enzymes (48 kDa). NI-type protein kinases from liver or hepatoma stimulated hepatoma nuclear and cytosolic poly(A) polymerases 4–6-fold. In contrast, the liver cytosolic 40- and 48-kDa poly(A) polymerases were stimulated only slightly or inhibited by similar units of the protein kinases. Antibodies produced in rabbits against purified hepatoma nuclear poly(A) polymerase reacted equally well with hepatoma nuclear and cytosolic enzyme but only 80% as well with the liver cytosolic 48-kDa poly(A) polymerase and not at all with liver cytosolic 40-kDa or nuclear 36-kDa enzymes. Anti-poly(A) polymerase antibodies present in the serum of a hepatoma-bearing rat reacted with hepatoma nuclear and cytosolic poly(A) polymerases to the same extent but only 40% as well with the liver cytosolic 48-kDa enzyme. From this value and the relatively low quantity of liver 48-kDa poly(A) polymerase, it is calculated that per unit weight of tissue, adult liver contains only 3% of the tumor poly(A) polymerase determinants which are immunogenic in an allogenic host. On the basis of the CNBr cleavage maps, immunological characteristics, and response to NI-type protein kinases, it is concluded that (a) hepatoma cytosol contains a single poly(A) polymerase that is identical with the hepatoma nuclear enzyme and (b) liver cytosol contains two distinct poly(A) polymerases, one (48 kDa) similar to but not identical with the hepatoma enzyme (48 kDa) and the other (40 kDa) possibly related to the major liver nuclear enzyme (36 kDa).

Several years ago, nuclear poly(A) polymerase from the rat tumor Morris hepatoma 3924A was shown to differ from the corresponding liver enzyme by a number of criteria (Rose & Jacob, 1976, 1979; Rose et al., 1978). Recent studies in our laboratory have demonstrated that nuclear poly(A) polymerases from the hepatoma (M_r 48 000) and liver (M_r 36 000–38 000) are structurally and immunologically distinct (Stetler & Jacob, 1984). These studies also demonstrated the existence of a minor (1% of total) liver nuclear poly(A) polymerase that was identical with the hepatoma nuclear enzyme with respect to immunological characteristics, molecular weight, and CNBr cleavage map. Because the same 48-kilodalton (kDa) enzyme was the major species isolated from fetal rat liver nuclei (Stetler & Jacob, 1985), this enzyme could be the product of an oncofetal gene which is expressed only to a limited extent in normal adult liver. However, poly(A) polymerase has also been identified in other cellular fractions including mitochondria (Jacob et al., 1972; Rose et al., 1975; Aujame & Freeman, 1976), microsomes (Wilke & Smellie, 1968; Rose

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& Jacob, 1975; Avramova et al., 1980), ribosomes (Bretthauer & Twu, 1971; Avramova et al., 1980), and cytosol (Tsiapolis et al., 1975; Rose et al., 1976; Nevins & Joklik, 1977). Because any of these enzymes might be related to the hepatoma and minor liver nuclear poly(A) polymerase, it is possible that instead of differential expression of the gene coding for a distinct "tumor/fetal-type" enzyme species, there could be translocation of this enzyme from nuclei to cytoplasm during cellular differentiation and from cytoplasm to nuclei during tumorigenesis. To clarify this issue, we have purified poly(A) polymerase from the cytosol of rat hepatoma and normal liver and have compared these enzymes to those from isolated nuclei.

Materials and Methods

Enzyme Purification. (A) Nuclear Poly(A) Polymerase. Poly(A) polymerase was purified from isolated nuclei of Morris hepatoma 3924A and normal rat liver by our published protocol (Rose & Jacob, 1976; Rose et al., 1978) except that the linear salt gradients used for elution of enzyme from phosphocellulose columns were expanded (Stetler & Jacob, 1984) and DNA covalently bound to diazotized [diazobenzyloxymethyl (DBM)] cellulose (described below) was utilized for the last chromatographic step instead of conventional DNA–cellulose.

(B) Cytosolic Poly(A) Polymerase. The supernatant fractions obtained after centrifugation of the tissue homogenate in hypertonic sucrose to sediment the nuclei were diluted to a final concentration of 0.44 M sucrose in 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5), 0.01 M $MgCl_2$, 0.025 M KCl, 0.5 mM dithiothreitol (DTT), and 0.1 mM ethylenediaminetetraacetic acid (EDTA) (Rose et al., 1976). Following centrifugation at 100000*g* for 90 min to remove mitochondria, microsomes, and lysosomes, cytosolic proteins were precipitated with $(NH_4)_2SO_4$ and prepared for DEAE-Sephadex column chromatography as described for nuclear enzymes (Rose & Jacob, 1976). Fractionation on DEAE-Sephadex columns and also the subsequent fractionations on phosphocellulose, hydroxylapatite, QAE-Sephadex, and DNA–DBM-cellulose columns were performed essentially as outlined for purification of nuclear poly(A) polymerase (Stetler & Jacob, 1984). Briefly, the cytosolic poly(A) polymerases from both tissues were collected in the 10 mM $(NH_4)_2SO_4$ wash fraction from the DEAE-Sephadex column (2.0 and 0.5 mL of gel/g of initial tumor and liver, respectively) and applied to phosphocellulose columns (60 and 40 mL/100 g of tumor and liver, respectively). Poly(A) polymerase was eluted with a linear KCl gradient from the phosphocellulose columns as a single activity peak (tumor, 270 mM KCl; liver, 370 mM KCl) and, after dialysis, applied to a QAE-Sephadex column (4 and 2 mL/100 g of tumor and liver). Poly(A) polymerase was collected in the 10 mM NaCl flow-through and wash fractions and immediately applied to DNA–DBM-cellulose columns (1.5 and 2.5 mL/100 g of liver and tumor, respectively). Poly(A) polymerase, eluted with a linear NaCl gradient as a single peak, was dialyzed against buffer containing 50% glycerol and stored at −70 °C.

Poly(A) polymerase activity was assayed as described (Rose & Jacob, 1976). Reactions were for 30 min at 37 °C. One unit of poly(A) polymerase activity represents 1 nmol of AMP incorporated into poly(A).

Protein Kinases. The protein kinases which separated from nuclear poly(A) polymerase during purification of the liver (phosphocellulose column) and hepatoma (hydroxylapatite) poly(A) polymerases were purified through the casein–Sepharose stage as described (Stetler et al., 1984a). One unit of protein kinase represents 1 nmol of the terminal phosphate of ATP transferred to casein under the conditions outlined.

Preparation of DNA–DBM-cellulose. Cellulose (Sigma Chemical Co., St. Louis, MO; type 100) was diazotized essentially as described (Christophe et al., 1982) for cellulose sheets. Briefly, the cellulose was suspended in *N,N*-dimethylformamide (1.75 mL/g of dry cellulose) containing 0.2 g of *N*-(3-nitrobenzyloxymethyl)pyridinium chloride (Sigma Chemical Co., St. Louis, MO) per milliliter, spread out into a glass dish, and incubated at 130–135 °C for 30 min. After the dish was cooled, the (nitrobenzyloxymethyl)cellulose was washed on a glass-fritted funnel with acetone until the eluate was clear and then several times with distilled water. The (nitrobenzyloxymethyl)cellulose was reduced to (aminobenzyloxymethyl)cellulose by stirring for 2 h in a solution consisting of 10% sodium dithionite and 50 mM sodium hydroxide. The (aminobenzyloxymethyl)cellulose was washed on a glass-fitted funnel with distilled water until the odor of dithionite was no longer detectable and washed 4 times with 5 volumes each of acetone and distilled water. The (aminobenzyloxymethyl)cellulose was activated to (diazobenzyloxymethyl)cellulose (DBM-cellulose) as described (Christophe et al., 1982) and then stirred at 4 °C for 60 h in 10 volumes of 25 mM sodium phosphate (pH 6.5) containing 2 mg/mL calf thymus DNA. To remove unbound DNA (usually less than 10%), the DNA–DBM-cellulose was washed at 4 °C with 50 mM Tris-HCl (pH 7.9) on a glass-fritted funnel until the A_{260} of the eluate was zero. To bind unreacted diazonium groups, the DNA–DBM-cellulose was stirred at 4 °C for 16 h in 10 volumes of 50 mM Tris-HCl (pH 7.9) containing 1 M glycine and 1% bovine serum albumin. Finally, the DNA–DBM-cellulose was washed extensively with 50 mM Tris-HCl (pH 7.9) containing 1.5 M NaCl until the A_{280} of the eluate was zero and stored at 4 °C in the same buffer containing 0.1% sodium azide. After each use, the DNA–DBM-cellulose was washed with the same buffer and reused over a period of 8 months with no loss of capacity.

Results

Purification of Poly(A) Polymerase from Cytosol and Nuclei of Rat Hepatoma and Liver. Poly(A) polymerase was purified from the cytosol fractions of the rat tumor Morris hepatoma 3924A and normal rat liver essentially by the protocol described (Rose & Jacob, 1976; Stetler & Jacob, 1984) for purification of nuclear poly(A) polymerases. Details of the procedure are given under Materials and Methods, and the results are summarized in Table I. After DEAE-Sephadex column chromatography, approximately twice as much poly(A) polymerase was recovered from hepatoma cytosol as from hepatom nuclei. In contrast, greater than 6 times as much activity was recovered from normal liver cytosol as from the corresponding nuclei. However, the poly(A) polymerase activity in either the nuclei or the cytosol from hepatoma was significantly greater than that in the corresponding liver fractions. Thus, approximately 17 times more nuclear enzyme activity and 5 times more cytosolic enzyme activity were recovered from hepatoma than from the corresponding liver fractions.

The activity recovered from liver nuclei was near the average value of several previous extractions (Rose & Jacob, 1976; Rose et al., 1976; Stetler et al., 1984b). However, since the initial report (Rose & Jacob, 1976) of purification of poly(A) polymerase from the nuclei of Morris hepatoma 3924A, the recovery of the nuclear enzyme from this tissue has improved dramatically. The hepatomas are now transplanted in our own

Table I: Purification of Poly(A) Polymerase from Cytosol and Nuclei of Rat Liver and Hepatoma

	poly(A) polymerase activity							
	units/100 g of tissue				units/mg of protein			
	hepatoma		liver		hepatoma		liver	
purification stage	nuclei	cytosol	nuclei	cytosol	nuclei	cytosol	nuclei	cytosol
DEAE-Sephadex	37458	72538	2255	13973	106	183	103	90
phosphocellulose	6606	12213	1002 (354)[a]	4788	607	401	204 (131)	200
hydroxylapatite	4825	777	495 (158)	1384	894	629	429 (397)	870
DNA–DBM-cellulose	3549	344	56 (35)	217	4628	1394	558 (497)	1375

[a] Two distinct liver nuclear poly(A) polymerases were separated by phosphocellulose chromatography (Stetler & Jacob, 1984). The numbers in parentheses are the values obtained during purification of the minor species.

laboratory and are virtually free of necrotic tissue. In previous studies, attempts to remove necrotic tissue delayed the extraction process considerably and resulted in reduced recovery and specific activity of the enzyme. A second factor that has contributed to greater recovery of purified enzyme with high specific activity has been the use of affinity columns composed of DNA covalently bound to diazotized (DBM) cellulose in the last step of purification. The DNA–DBM-cellulose column was superior to the DNA–cellulose column in a number of ways. First, the DNA–DBM-cellulose was very stable and could be used repeatedly with no apparent loss of capacity. Second, a greater quantity of DNA could be bound to the diazotized cellulose so that the capacity of the column for poly(A) polymerase was substantially improved. Third, the greater capacity allowed the use of smaller columns which resulted in concentration of poly(A) polymerase. This apparently stabilized the enzyme so that greater yields and specific activities were obtained. Thus, from the DNA–DBM-cellulose column, almost 75% of the applied hepatoma poly(A) polymerase was recovered with a specific activity of greater than 4500 units (mg of protein)$^{-1}$ (30 min)$^{-1}$. In contrast, yields of only 20–30% and specific activities ranging from 400 to 900 units/mg of protein were usually obtained with conventional DNA–cellulose (unpublished results).

Polyacrylamide Gel Electrophoresis and Immunoblot of Purified Poly(A) Polymerases. As shown in Figure 1, the purified enzyme from hepatoma cytosol exhibited a single polypeptide band after polyacrylamide gel electrophoresis under denaturing conditions which corresponded to the purified hepatoma nuclear enzyme (M_r 48 000). The highly purified liver cytosol preparation contained two major polypeptides, one of M_r 48 000 (48 kDa) and the other of M_r 40 000 (40 kDa). The 48-kDa polypeptides of all three purified enzyme preparations (liver cytosol, hepatoma nuclei, and hepatoma cytosol), but not the 40-kDa liver cytosol polypeptide, reacted with antibodies produced in rabbits immunized with hepatoma nuclear poly(A) polymerase (Figure 2). These results indicated that the hepatoma and liver cytosol 48-kDa polypeptides were at least immunologically related to hepatoma nuclear poly(A) polymerase. The anti-hepatoma nuclear poly(A) polymerase antibodies are known to react with all of the CNBr cleavage fragments of the hepatoma nuclear enzyme (Stetler & Jacob, 1984), indicating a distribution of antigenic determinants throughout the enzyme molecule. Hence, the 40-kDa liver cytosolic polypeptide was not merely a degradation product of the larger polypeptide, since only the 48-kDa polypeptide formed an immune complex with the antibodies.

Separation of Liver Cytosolic Poly(A) Polymerases by Phosphocellulose Chromatography. The major (36–38 kDa) and minor 48 kDa) poly(A) polymerases of liver nuclei could be separated by either phosphocellulose (PC) column chromatography or gel filtration (Stetler & Jacob, 1984). However, after gel filtration of the liver nuclear enzymes, poly(A)

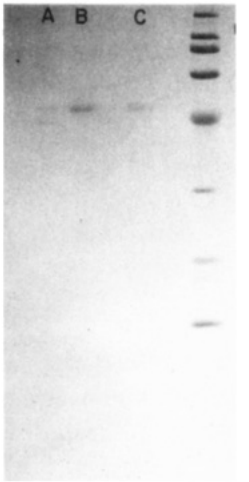

FIGURE 1: Polyacrylamide gel electrophoresis of purified cytosolic poly(A) polymerases. Poly(A) polymerases (3 μg) purified through the DNA–DBM-cellulose stage from liver cytosol (track A), hepatoma cytosol (track B), and hepatoma nuclei (track C) were subjected to polyacrylamide gel (12%) electrophoresis under denaturing (sodium dodecyl sulfate) conditions (Rose et al., 1981) and stained with Coomassie blue. Molecular weight markers (5 μg of each), electrophoresed on a parallel track shown at the extreme right, were myosin (M_r 200 000), β-galactosidase (M_r 130 000), phosphorylase *b* (M_r 97 400), ovalbumin (M_r 43 000), carbonic anhydrase (M_r 30 000), trypsin inhibitor (M_r 20 100), and α-lactalbumin (M_r 14 400).

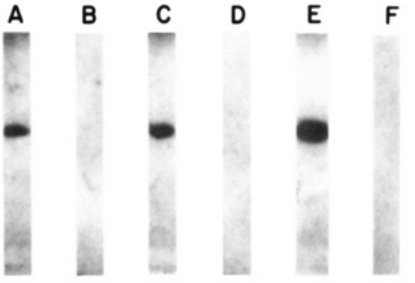

FIGURE 2: Immunoblot of purified poly(A) polymerases. Poly(A) polymerases purified through the DNA–DBM-cellulose stage from hepatoma nuclei (5 μg; tracks A and B), liver cytosol (10 μg; tracks C and D), and hepatoma cytosol (10 μg; tracks E and F) were electrophoresed under denaturing (sodium dodecyl sulfate) conditions (Rose et al., 1981) on a linear gradient (2.5–16%) polyacrylamide gel and transferred electrophoretically (Stetler et al., 1981) to freshly activated (*Christopher* et al., 1982) DBM paper. Tracks A, C, and E were incubated with rabbit anti-hepatoma nuclear poly(A) polymerase antiserum. Tracks B, D, and F were incubated with serum from a nonimmunized rabbit. Immune complexes were detected with ^{125}I-protein A and autoradiography as described (Stetler et al., 1981).

polymerase activity could only be recovered by addition of NI-type protein kinase to the column fractions (Stetler & Jacob, 1984). In the absence of the kinase, no poly(A) polymerase activity could be detected. A similar loss of enzyme activity was observed when the highly purified liver cytosolic poly(A) polymerase was subjected to gel filtration (Sephacryl

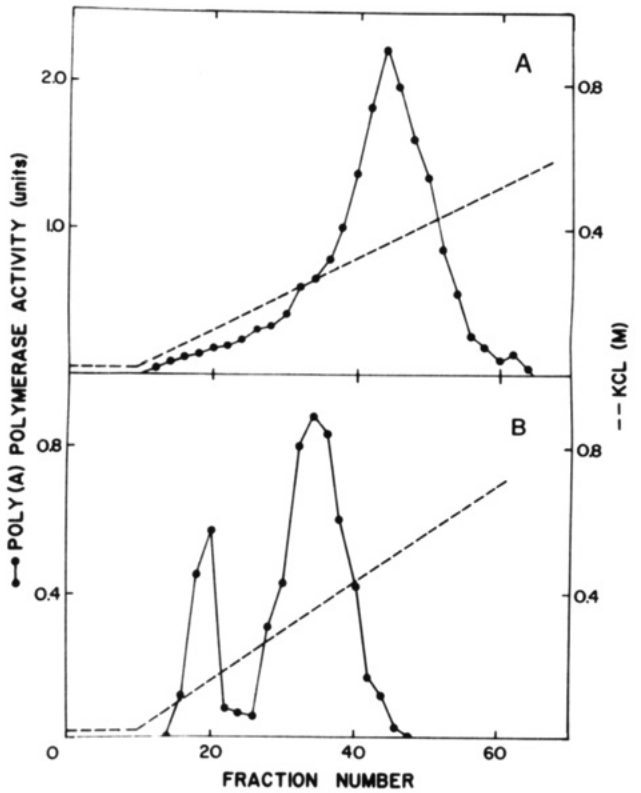

FIGURE 3: Phosphocellulose column chromatography of liver cytosolic poly(A) polymerase. Liver cytosolic poly(A) polymerase from the DEAE-Sephadex column (panel A) or from the DNA–DBM-cellulose column (panel B) was fractionated by phosphocellulose chromatography essentially as described under Materials and Methods. The DEAE-Sephadex-purified poly(A) polymerase (1×10^4 units) was applied to a 40-mL phosphocellulose column, and 5-mL fractions were collected. The DNA–DBM-purified enzyme (130 units) was applied to a 2-mL phosphocellulose column, and 0.3-mL fractions were collected. Recovery from the two columns (A and B) was approximately 34% and 60%, respectively. Samples (30 μL) of every second fraction were then assayed for poly(A) polymerase activity.

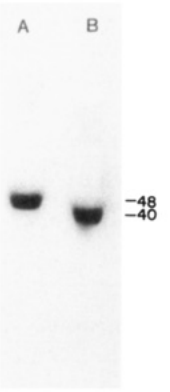

FIGURE 4: Polyacrylamide gel electrophoresis of liver cytosolic poly(A) polymerase. Liver cytosolic poly(A) polymerase (5 μg) from the second phosphocellulose column (see Figure 3, panel B) were electrophoresed under denaturing conditions on a linear gradient (2.5–16%) polyacrylamide gel and stained with Coomassie blue. Molecular weights, indicated on the right ($\times 10^{-3}$) were calculated from markers (see Figure 1) electrophoresed on a parallel track. Tracks A and B are the second and first poly(A) polymerase activity peaks, respectively, from the second phosphocellulose column (see Figure 3, panel B).

Table II: Reaction of Liver and Hepatoma Cytosolic Poly(A) Polymerases with Rabbit Anti-Hepatoma Nuclear Poly(A) Polymerase Antibodies

source of poly(A) polymerase	immunoreactivity (cpm ± SEM)[a]
liver cytosol (40 kDa)	17 ± 9
liver cytosol (48 kDa)	1221 ± 60
hepatoma nuclei (48 kDa)	1463 ± 21
hepatoma cytosol (48 kDa)	1509 ± 37

[a] The liver cytosolic poly(A) polymerases (0.1 μg) from the second phosphocellulose column (Figure 3, panel B) and the hepatoma enzymes (0.1 μg) from the DNA–DBM columns were adsorbed to microtiter wells and incubated with serum from a rabbit that had been immunized with hepatoma nuclear poly(A) polymerase. Immune complexes were detected with ^{125}I-protein A as described earlier (Stetler et al., 1982). Results are the mean of triplicate reactions and are expressed as the cpm of ^{125}I-protein A bound to the wells ± SEM.

S-200). However, contrary to the nuclear poly(A) polymerases, the cytosolic enzyme inactivation by gel filtration was not reactivated by either liver or hepatoma NI-type protein kinase (not shown).

Because of the loss of activity during gel filtration, an attempt was made to separate two liver cytosolic poly(A) polymerases by rechromatography on PC. Only one major activity peak (eluted at 0.37 M KCl) was apparent when the partially purified liver cytosolic poly(A) polymerase was originally chromatographed on this column (Figure 3, panel A). However, when the highly purified liver cytosolic poly(A) polymerase from the DNA–DBM-cellulose column was chromatographed on PC, two distinct activity peaks eluted at 0.14 and 0.36 M KCl (Figure 3, panel B). Other proteins present in the partially purified enzyme preparation might have prevented separation of the two cytosolic poly(A) polymerase during chromatography on the first PC column. Protein kinase was present in the enzyme preparation applied to the first PC column and eluted at 0.45 M KCl (not shown). The affinity of poly(A) polymerase for the protein kinase may have been responsible for the higher salt concentration (0.37 M) required for elution of the partially purified polymerase compared to the concentration (0.14 M) required for elution of the highly purified polymerase which contained no protein kinase. Regardless, analysis of the second PC column fractions by polyacrylamide gel electrophoresis under denaturing conditions revealed that the first activity peak contained a single polypeptide of M_r 40 000 while the second peak contained a single polypeptide of M_r 48 000 (Figure 4). As would be expected from the immunoblot analysis (Figure 2), the 48-kDa liver cytosolic poly(A) polymerase reacted with anti-hepatoma nuclear poly(A) polymerase antibodies while the 40-kDa enzyme did not (Table II). These results confirmed that liver cytosol contained two distinct poly(A) polymerases.

CNBr Cleavage Maps. To investigate whether the nuclear and cytosolic poly(A) polymerases were structurally related, the CNBr cleavage maps of the enzymes were compared (Figure 5). Hepatoma nuclear and cytosolic poly(A) polymerase polypeptides (48 kDa) produced essentially identical cleavage maps (tracks A and B). In contrast, the 48-kDa liver cytosolic enzyme produced a CNBr cleavage pattern that, although quite similar, was significantly different from that of the hepatoma 48-kDa enzymes (track C). Thus, of the seven smaller polypeptide fragments of the hepatoma poly(A) polymerases (arrows between tracks A and B), six were present in the map of the liver cytosolic 48-kDa enzyme (arrows between tracks B and C). However, the largest of the seven fragments (star) was absent in the liver enzyme cleavage pattern and was replaced by two smaller fragments (asterisks). Other differences were apparent in the size of the larger, partial digestion fragments; two bands (×) were present in the map of the liver cytosolic 48-kDa enzyme that were absent in the cleavage patterns of the hepatoma enzymes. The CNBr cleavage map of the minor liver nuclear poly(A) polymerase (48 kDa) was previously shown (Stetler & Jacob, 1984) to be identical with the map of the hepatoma nuclear enzyme.

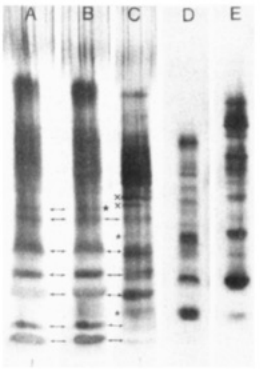

FIGURE 5: CNBr cleavage maps of poly(A) polymerases. Purified poly(A) polymerases (20 μg) were subjected to polyacrylamide gel (12%) electrophoresis under denaturing conditions. The enzyme polypeptides, located by Coomassie blue staining of parallel tracks containing 3 μg of enzyme, were cut from the gel and subjected to CNBr cleavage as described (Stetler & Jacob, 1984). The cleaved polypeptide-containing gel slices were then placed on another polyacrylamide (12%) slab gel and electrophoresed under denaturing conditions. Peptides were visualized by silver staining (UpJohn Diagnostic, Kalamazoo, MI). Enzymes were hepatoma nuclear 48-kDa (track A), hepatoma cystolic 48-kDa (track B), liver cytosolic 48-kDa (track C), liver nuclear 36-kDa (track D), and liver cytosolic 40-kDa (track E) poly(A) polymerases. Symbols between tracks are explained in the text.

As was expected from the results of the immunoblot (Figure 2), the 40-kDa liver cytosolic polypeptide exhibited a cleavage pattern that was distinct from those of the 48-kDa polypeptides (Figure 5, track E). However, the 40-kDa polypeptide cleavage map appeared quite similar to that of the 36-kDa liver nuclear poly(A) polymerase when the difference in molecular weight prior to cleavage was considered (track D). Thus, hepatoma appears to contain a single poly(A) polymerase species which is present in both the nuclear and cytosolic fractions. In contrast, liver cytosol contains two poly(A) polymerase species, one of which is similar to, but not identical with, hepatoma enzyme and the minor liver nuclear enzyme, whereas the other may be related to the major enzyme from liver nuclei.

Effect of Phosphorylation on Poly(A) Polymerase Activity. We have previously reported (Rose & Jacob, 1979; Stetler et al., 1984a) that NI-type protein kinase activity is separated from both hepatoma and liver nuclear poly(A) polymerases during purification of the polymerizing enzymes. Although the kinases separated from hepatoma and liver nuclear poly(A) polymerases are distinct enzymes (Stetler et al., 1984a), they are both capable of stimulating either hepatoma or liver nuclear poly(A) polymerase activity in vitro. Protein kinase activity was also separated from the cytosolic poly(A) polymerases during phosphocellulose chromatography (not shown). However, the failure of both liver and tumor NI-type protein kinases to reactivate liver cytosolic poly(A) polymerase that had been subjected to gel filtration (see Separation of Liver Cytosolic Poly(A) Polymerases by Phosphocellulose Chromatography) suggested that cytosolic poly(A) polymerases, unlike the nuclear enzymes, might not be stimulated by these kinases. Hence, it was of interest to determine whether cytosolic poly(A) polymerases that had not been subjected to gel filtration would respond to protein kinase. The effect of NI-type protein kinase from hepatoma and liver on the poly(A) polymerase activities was determined by adding a constant amount of either kinase to similar quantities of each poly(A) polymerase. As shown in Table III, the NI-type protein kinase separated from hepatoma nuclear poly(A) polymerase and subsequently purified (Stetler et al., 1984a) was capable of

Table III: Effect of NI-Type Kinases on Activity of Purified Nuclear and Cytosolic Poly(A) Polymerases

source of poly(A) polymerase[a]	poly(A) polymerase act. in presence of protein kinase (% of control)[b]	
	hepatoma NI protein kinase	liver NI protein kinase
hepatoma nuclei (48 kDa)	465	395
hepatoma cytosol (48 kDa)	600	478
liver nuclei (36 kDa)	326	297
liver cytosol (48 kDa)	145	75
liver cytosol (40 kDa)	57	96

[a] DNA–DBM-cellulose-purified hepatoma poly(A) polymerases, or liver enzymes purified further by phosphocellulose chromatography, were assayed, in triplicate, in the standard poly(A) polymerase reaction in the absence and presence of 0.025 unit of NI-type protein kinase purified (Stetler et al., 194a) from hepatoma or liver nuclei. [b] Results are expressed as (the mean activity in the presence of protein kinase/the mean activity in the absence of protein kinase) × 100. Control poly(A) polymerase activity (no added protein kinase) was 0.75 ± 0.05 to 1.03 ± 0.07 units ± SEM.

stimulating the 48-kDa poly(A) polymerase from hepatoma cytosol (6-fold) and nuclei (4.8-fold). Similarly, NI-type protein kinase purified from liver nuclei activated hepatoma nuclear (4-fold) and cytosolic (4.8-fold) poly(A) polymerase. These results further supported the contention that the hepatoma cytosolic and nuclear 48-kDa poly(A) polymerase are identical. As reported earlier (Stetler et al., 1984a), the major liver nuclear poly(A) polymerase (36 kDa) was also stimulated by the tumor (3.3-fold) and liver (3.0-fold) NI-type protein kinases (Table III). In contrast, neither of the two liver cytosolic poly(A) polymerases was significantly activated by the kinases. In fact, the 48-kDa liver cytosolic poly(A) polymerase was actually inhibited (25%) by the liver kinase while the 40-kDa poly(A) polymerase was inhibited (43%) by the hepatoma kinase. That phosphorylation of the poly(A) polymerases has indeed occurred was confirmed by substituting [γ-^{32}P]ATP for the [^{3}H]ATP in the reaction mixture followed by analysis of the trichloroacetic acid precipitated proteins. In all cases, radioactive phosphate was incorporated into poly(A) polymerase polypeptides (not shown). Similar results were obtained when partially purified cytosolic protein kinases [separated from poly(A) polymerase by PC chromatography] were used (not shown). These data provided further evidence that the liver cytosolic 48- and 40-kDa poly(A) polymerases are distinct from each other and, although structurally related to the hepatoma and major liver nuclear poly(A) polymerases, respectively, are not identical with these enzymes.

Reaction of 48-kDa Poly(A) Polymerases with Serum from an Immunized Rabbit and from a Hepatoma-Bearing Rat. Antibodies capable of reacting with hepatoma nuclear poly(A) polymerase (48 kDa) but not with the adult liver major nuclear poly(A) polymerase (36–38 kDa) have been detected in the sera of rats bearing a variety of transplanted Morris hepatomas and a mammary adenocarcinoma (Stetler et al., 1981) as well as primary hepatomas induced by *N,N*-dimethyl-*p*-(*m*-tolylazo)aniline (Stetler et al., 1984b). Therefore, it was of interest to determine whether these antibodies would interact with cytosolic 48-kDa poly(A) polymerases of hepatoma and normal liver. As shown in Table IV, antibodies in the serum of a hepatoma-bearing rat reacted with equal quantities of hepatoma nuclear and cytosolic enzymes to a similar extent. In contrast, as observed previously (Stetler & Jacob, 1984), the antibodies had almost no affinity for the liver major nuclear enzyme (36–38 kDa). Further, the antibodies reacted with the liver cytosolic 48-kDa enzyme only about 40% as well as with the hepatoma enzymes. Thus, the liver cytosolic 48-kDa

Table IV: Reaction of Poly(A) Polymerases with Anti-Poly(A) Polymerase Antibodies in Serum of a Hepatoma-Bearing Rat[a]

source of poly(A) polymerase	immunoreactivity (cpm ± SEM)
hepatoma nuclei	3991 ± 98
hepatoma cytosol	4324 ± 35
liver nuclei (36 kDa)	0 ± 6
liver cytosol (48 kDa)	1623 ± 142
liver cytosol (40 kDa)	0 ± 15

[a] 0.6 μg of the purified poly(A) polymerase indicated was adsorbed to polystyrene microtiter wells and incubated with antisera. Antibody binding was quantitated with ^{125}I-protein A (Stetler et al., 1982). Antiserum was from a rat that had borne transplanted Morris hepatoma 9618A for 9 weeks (Stetler et al., 1981).

poly(A) polymerase possessed less than half of the antigenic determinants present on the hepatoma enzymes which were recognized by the antibodies produced in the primary tumor-bearing host. Similar results were obtained with serum from a rabbit immunized with hepatoma nuclear poly(A) polymerase except that the reaction with the liver cytosolic 48-kDa poly(A) polymerase was 80% of that with the hepatoma enzymes (see Table II). Thus, the rat liver cytosolic 48-kDa poly(A) polymerase possessed more than three-fourths of the hepatoma enzyme antigenic determinants that were recognized as "foreign" by a xenogenic host (rabbit). The data with both antisera are consistent with the structural studies (Figure 5).

Discussion

On the basis of molecular weight, CNBr cleavage pattern, reactivity with antipoly(A) polymerase antibodies, and response to NI-type protein kinases (see Table V), it can be concluded that the hepatoma cytosolic poly(A) polymerase is identical with the hepatoma nuclear enzyme. In contrast to the single poly(A) polymerase species in hepatoma nuclei and cytosol, adult liver contains two poly(A) polymerases in each of these cellular fractions which can be distinguished from each other by at least one of these criteria (see Table V). Previous studies have shown that the liver nuclear 48-kDa enzyme is identical with the hepatoma nuclear 48-kDa enzyme (Stetler & Jacob, 1984). Further, liver cytosolic 48-kDa enzyme was demonstrated (Figure 5) to be similar to, but not identical with, the hepatoma enzymes and distinct from the liver nuclear 36-kDa species. These data indicate that the liver nuclear 36–38-kDa and 48-kDa poly(A) polymerases and the liver cytosolic 48-kDa species are distinct gene products (see Table V). The liver nuclear 36–38-kDa and cytosolic 40-kDa poly(A) polymerases differed in molecular weight and response to protein kinase as well as in CNBr cleavage patterns. However, differences in the CNBr cleavage patterns of these two enzymes could have been the result of the difference in initial molecular weight. Hence, at this time, we cannot rule out the possibility that these two enzymes are differentially processed products of the same gene. Whether any one of these enzymes is related to mitochondrial or microsomal poly(A) polymerase is not known.

The level of poly(A) polymerase in the nucleus is known to be elevated in rat hepatoma compared to normal liver (Rose & Jacob, 1976). Others have reported (Trangas et al., 1984) greater total poly(A) polymerase activity in human leukemic lymphocytes compared to normal lymphocytes. In the present study, both nuclear and cytosolic poly(A) polymerase activities were elevated in hepatoma relative to that in normal liver (Table I). Thus, per 100 of tissue, hepatoma nuclei and cytosol contained a combined total of almost 110×10^3 units of poly(A) polymerase activity with 34% of it being localized in the nucleus. In contrast, the same fractions from normal liver contained a combined total of only 16×10^3 units with only 14% of it present in the nucleus. Recently, we reported (Stetler & Jacob, 1985) that fetal rat liver nuclear poly(A) polymerase (48 kDa) is structurally and immunologically identical with the hepatoma nuclear enzyme. The levels of fetal poly(A) polymerase activity were also comparable to those of hepatoma enzyme. Of a combined total of fetal cytosolic and nuclear poly(A) polymerase activity of 55×10^3 units per 100 g of tissue, 32% was recovered from nuclei (unpublished results; see Table V). This elevation of poly(A) polymerase activity in both the nucleus and cytosol of hepatoma and fetal liver, and the presence of approximately 30% in the nuclear fraction, does not appear to be merely a characteristic of actively dividing tissue. In preliminary studies, regenerating rat liver (18-h post-partial hepatectomy) was found to contain a combined total of nuclear and cytosolic poly(A) polymerase activity of only 7×10^3 units per 100 g of tissue with 91% recovered from nuclei (Stetler & Jacob, 1985; unpublished results).

Table V: Comparison of Hepatoma Nuclear Poly(A) Polymerase to Poly(A) Polymerases from Other Tissues and Cellular Fractions

	hepatoma		adult liver		fetal liver[i]	
	nuclei	cytosol	nuclei[h]	cytosol	nuclei	cytosol
act. relative to hepatoma nuclei[a]	1.00	1.94	0.06	0.37	0.48	1.00
no. of enzyme species	1	1	2	2	1	ND[j]
polypeptide $M_r \times 10^{-3}$ [b]	48	48	36–38/48	40/48	48	ND
% of total[c]	100	100	90–99/1–10	50/50	100	ND
relative immunoreactivity with hepatoma-bearing rat anti-poly(A) polymerase antibodies[d]	1.0	1.1	0.0/1.0	0.0/0.4	1.0	ND
relative quantity of immunoreactive enzyme antigenic determinants[e]	1.00 (1.00)	2.13	0.00/0.01 (0.03)	0.00/0.07	0.48	ND
response to protein kinase NI[f] from						
liver	↑	↑	↑/↑	↔/↓	ND	ND
hepatoma	↑	↑	↑/↑	↓/↔⁺	ND	ND
CNBr cleavage pattern[g]	7 major fragments	=	≠/=	≠/≃	=	ND

[a] Enzyme activities are expressed relative to hepatoma nuclear enzyme activity (1.00 = 37458 units/100 g of tissue). [b] Determined by polyacrylamide gel electrophoresis under denaturing (sodium dodecyl sulfate) conditions. [c] Values given for adult liver nuclear enzymes were determined by immunological techniques (Steler & Jacob, 1984). Values given for adult liver cytosolic enzymes were based on the presence of equal quantities of 40- and 48-kDa polypeptides after purification. [d] Data from Table IV are expressed relative to results with the hepatoma nuclear enzyme. Values given for the immunoreactivity of adult and fetal liver 48-kDa enzymes are from unpublished data. [e] Relative quantity of immunoreactive enzyme antigenic determinants = relative activity × percent of total × relative immunoreactivity with serum from a tumor-bearing rat. Numbers in parentheses are values of cytosolic and nuclear enzymes combined (relative to the hepatoma nuclear + cytosolic enzymes taken as 1.00). [f] Abbreviations: ↑, activated; ↓, inhibited; ↔, no effect; ↔⁺, slightly activated. [g] Abbreviations: =, identical; ≠, distinct; ≃, similar. [h] Values derived from Table IV, previous studies (Stetler & Jacob, 1984; Stetler et al., 1984a), and unpublished data. [i] Values derived from previous studies (Stetler & Jacob, 1985) and unpublished data. [j] ND = not determined.

Hence, although nuclear poly(A) polymerase activity increased approximately 3-fold in regenerating liver compared to normal liver, the cytosolic enzyme was reduced to approximately 5% of the normal level. However, the level of the 48-kDa enzyme species in the nucleus, determined immunologically (Stetler & Jacob, 1985), did not increase over the level detected in normal liver nuclei. These data rule out translocation of the cytosolic enzyme to the nucleus during liver regeneration. The apparent decrease in cytosolic poly(A) polymerase during liver regeneration might be due to a dramatic increase in poly(A)-degrading nuclease that is known to be present in some poly(A) polymerase preparations (Jacob & Rose, 1983; Muller et al., 1983). Nevertheless, the different levels and types of poly(A) polymerase present in nuclei and cytosol of liver cells in varying states of differentiation indicate a role for these enzymes in the regulation of mRNA processing.

The 48-kDa tumor-type poly(A) polymerase represents the major, if not the only, enzyme species in fetal rat liver nuclei (Stetler & Jacob, 1985). It occurs as a very minor enzyme in normal adult (Stetler & Jacob, 1984) and regenerating (Stetler & Jacob, 1985) liver nuclei, as well as in normal adult kidney and spleen nuclei (unpublished results). These observations indicate that this enzyme is the product of an oncofetal gene. Although normal rat liver cytosol contained a similar 48-kDa poly(A) polymerase, it was not identical with the tumor enzyme by several criteria. Thus, the presence of only minor quantities of tumor/fetal-type 48-kDa poly(A) polymerase in normal rat liver nuclei is not due to translocation of the majority of the 48-kDa enzyme from the nucleus to cytosol during development from fetal to adult liver.

On the basis of the nearly equal quantities of 40- and 48-kDa liver cytosolic enzyme polypeptides (Figure 1), the 48-kDa enzyme constitutes only about 50% of the liver cytosolic poly(A) polymerase. However, this protein is only 40% as immunoreactive as the hepatoma enzyme with respect to antibodies produced by a tumor-bearing host (Table IV). Thus, normal liver cytosol and nuclei contain only 3% of the immunoreactive enzyme antigenic determinants of a comparable quantity of hepatoma (see Table V). Therefore, the production of anti-poly(A) polymerase antibodies by tumor-bearing hosts (Stetler et al., 1981) appears to be in response to the dramatic increase in quantity of immunogenic enzyme.

The response of the various poly(A) polymerases described here to exogenous NI-type protein kinase deserves some comment. Protein kinase activity is separated from hepatoma and liver nuclear poly(A) polymerases during purification, and nuclear poly(A) polymerase from either tissue is stimulated by readdition of either kinase (Ross & Jacob, 1979; Stetler et al., 1984a). Separation of protein kinase activity was also achieved in the present investigation during purification of cytosolic poly(A) polymerases (data not shown). The structural and/or functional relationship of the cytosolic protein kinases to those from the nucleus has not been firmly established. However, preliminary studies have shown that the responses of the poly(A) polymerases described here to the partially purified cytosolic kinases are similar to the responses observed to the nuclear kinases (not shown). The relative lack of activation of liver cytosolic poly(A) polymerases by the protein kinases even though phosphorylation occurred suggests that these enzymes are not subject to the same controls which govern the liver nuclear poly(A) polymerases. Thus, it is possible that nuclear and cytosolic poly(A) polymerases may respond differently to the various external stimuli that affect these enzyme activities [see Jacob & Rose (1983)].

Finally, the functional significance of the presence of four distinct species of poly(A) polymerase in the nuclei and cytosol of normal liver compared to just one species in hepatoma is not known. It is possible that each enzyme might polyadenylate specific classes of mRNA. Alternatively, the primary function(s) of one or more of the normal liver enzymes might be something other than the addition of adenylate tracts to RNA. Further studies are required to throw light on these issues.

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