Comparison of Endogenous and Exogenous RNA Primers of Poly(U) Polymerase in Rat Hepatic Ribosomes

By T. TERRY HAYASHI and KATHLEEN MacFARLANE

Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Magee-Womens Hospital, Forbes and Halket Streets, Pittsburgh, PA 15213, U.S.A.

(Received 22 August 1978)

The present work indicates that RNA primer requirements for poly(U) polymerase in the free ribosomes of the rat liver depend upon the degree of enzyme purification. The poly(U) polymerase activity obtained from a crude free ribosomal preparation was compared with the enzymic activity of a partially purified enzyme. After preliminary purification, the enzyme was fractionated by chromatography on Sephadex G-150 and CM-cellulose. Our results demonstrate the presence of several forms of poly(U) polymerase activities, some requiring exogenous RNA and others possessing their own endogenous primer RNA.

There have been many reports on adenylate-rich sequences in rapidly labelled RNA species (Edmonds & Abrams, 1960; Darnell *et al.*, 1971; Lim & Canelakis, 1970; Lee *et al.*, 1971; Edmonds *et al.*, 1971), but recent work has also demonstrated the occurrence of uridylate-rich sequences in heterogeneous rRNA and mRNA (Molloy *et al.*, 1974; Korwek *et al.*, 1976). Poly(U) polymerase activity (UTP-RNA uridylyl-transferase, EC 2.7.7.-) has been reported in various cytoplasmic and microsomal preparations (Wilkie & Smellie, 1968*a,b*; Klemperer, 1963; Wykes & Smellie, 1966; Burdon & Smellie, 1961; Hozumi *et al.*, 1975; Brishammar & Juntti, 1975; Villarreal & Holland, 1974; Burdon, 1963); more recently Milchev & Hadjiolov (1978) have described poly(U) polymerase in the cytoplasmic ribosomes of Ehrlich ascites-tumour cells. The present study reports the presence of similar poly(U) polymerase activity localized mainly in the free ribosomes of rat liver. In addition, after preliminary purification, the enzyme preparation was further fractionated by column chromatography on Sephadex G-150 and CM-cellulose. Our results indicate the presence of several forms of poly(U) polymerase activity, some requiring exogenous RNA and others possessing their own endogenous primer RNA.

Materials and Methods

Materials

[³H]UTP and other ³H-labelled nucleotides were obtained from Schwarz-Mann, Orangeburg, NY, U.S.A.; [α -³²P]UTP and [γ -³²P]UTP were purchased from ICN, Irvine, CA, U.S.A. Dowex 1(X8; 200–400 mesh) was procured from D & P Minerals Co. Analytical-grade reagents were utilized.

Isolation of ribosomes

Male Sprague-Dawley rats (Zivic Miller, Pittsburgh, PA, U.S.A.), kept without food overnight, weighing between 170 and 180 g were utilized for the study. The animals were killed by cervical dislocation and the livers were homogenized in 2 vol. of 0.25 M-sucrose in TKM buffer, pH 7.6 (0.05 M-Tris/HCl/0.025 M-KCl/0.5 mM-MgCl₂) containing 0.1 vol. of ribonuclease inhibitor (Blobel & Potter, 1967*a*). The homogenate was prepared with a Potter-Elvehjem homogenizer at 1725 rev./min; the post-mitochondrial supernatant was obtained by centrifuging the homogenate for 10 min at 17000 g. A portion (1.0 ml) of the post-mitochondrial supernatant was layered over 3.0 ml of 2.0 M-sucrose in TKM buffer in a cellulose nitrate tube. 0.25 M-Sucrose in TKM buffer was added to a total volume of 10 ml and centrifuged for 24 h in a Spinco no. 40 rotor at 105000 g (Hayashi & Kazmierowski, 1972). The resulting pellet contained the free ribosomes, and the membrane-bound ribosomes remained above the interface (Blobel & Potter, 1967*b*). The supernatant was decanted and homogenized, and the membrane-bound ribosomes were pelleted by centrifuging for 18 h in a Spinco no. 40 rotor at 40000 rev./min.

Assay of poly(U) polymerase activity

The reaction mixture contained 50 μ mol of Tris buffer, pH 8.5, 10 μ mol each of cysteine and MgCl₂, 15 μ Ci of [³H]UTP (specific radioactivity 100 μ Ci/ μ mol) and 0.1 ml of free ribosomal suspension; the total volume of reaction was 0.7 ml. After a 40 min incubation at 25°C, the reactions were stopped by adding 0.10 ml of cold 0.10 M-sodium pyrophosphate, pH 7.0, containing 2.0 mg of yeast RNA/ml and 2.0 mg of bovine serum albumin/ml (Roeder &

Rutter, 1970). Sodium dodecyl sulphate (5%, w/v). (0.1 ml) and 2 ml of cold 10% (w/v) trichloroacetic acid with 0.04 M-sodium pyrophosphate were then added. The resulting precipitate was collected on Whatman GF/C filters, washed eight times with 5 ml of cold 5% trichloroacetic acid containing 0.04 M-pyrophosphate and washed three times with 95% (v/v) ethanol. The radioactivity was determined in a liquid-scintillation counter by placing the dried filter in a counting vial containing 10.0 ml of Bray's solution (Bray, 1960). The amount of RNA in the ribosome fractions was measured by using a modified Schmidt-Tannhauser technique (Fleck & Munro, 1962). The protein was determined by the method described by Lowry *et al.* (1951).

Partial purification of poly(U) polymerase

Free ribosomes were suspended for 1 h in 1.0 ml of high-salt EDTA buffer (0.02 M-Tris/HCl/0.25 M-sucrose/0.5 M-KCl/0.01 M-EDTA/0.1 mM-dithiothreitol, pH 8.0). The ribosomal preparation was sonicated in a Branson model W185 Sonifier for 3 min in 10 s bursts. The resulting stripped free ribosomes were removed by centrifugation at 105000g for 1 h. The supernatant was dialysed overnight against 2 litres of buffer A (0.02 M-Tris/HCl/0.50 mM-EDTA/0.1 mM-dithiothreitol, pH 8.0).

Then 100%-saturated $(\text{NH}_4)_2\text{SO}_4$ solution was added to the supernatant to yield a 30%-saturated $(\text{NH}_4)_2\text{SO}_4$ solution and the resulting precipitate was removed by centrifugation at 17000g for 10 min. The 30%-saturated- $(\text{NH}_4)_2\text{SO}_4$ supernatant was

adjusted to 45% saturation and kept for 20 min at 0°C. The precipitate after centrifugation was dissolved in buffer A, pH 7.4. Protamine sulphate (final concentration 30 µg/ml) was added, and the mixture was incubated for 20 min at 0°C and then centrifuged at 17000g for 10 min. An equal volume of 100%-saturated $(\text{NH}_4)_2\text{SO}_4$ was added to the supernatant, which was then incubated for 20 min at 0°C. The protein was pelleted by centrifugation (17000g for 10 min) and dissolved in 8.0 ml of 0.05 M- $(\text{NH}_4)_2\text{SO}_4$ in buffer B (0.02 M-Tris/HCl/0.5 mM-EDTA/0.1 mM-dithiothreitol/5.0 mM-MgCl₂, pH 7.4). The protein solution was dialysed overnight against 0.05 M- $(\text{NH}_4)_2\text{SO}_4$ in buffer B, pH 7.4.

The enzyme preparation was further fractionated by column chromatography with Sephadex G-150 and CM-cellulose.

Poly(U) polymerase activity in isolated ribosomes

Table 1 indicates that the reaction has a requirement for cysteine and Mg^{2+} . Dithiothreitol effectively substituted for cysteine, but β -mercaptoethanol was only half as efficient. The replacement of [³H]UTP with [³H]UMP, [³H]UDP or with other ³H-labelled nucleoside triphosphates resulted in a marked decrease in the incorporation of the labelled compound, indicating the specificity of this reaction for UTP. The addition of other non-radioactive nucleoside triphosphates had no significant effect; there was no measurable change with actinomycin D, deoxyribonuclease or α -amanitin, indicating that the present system is not a DNA-dependent RNA polymerase. The inhibition observed with pyrophosphate suggests that the enzymic reaction does involve the release of pyrophosphate. Both Pronase and ribonuclease had a profound inhibiting effect upon the degree of UMP incorporation, and a moderate inhibition of the reaction was observed with sucrose and with EDTA.

The optimum Mg^{2+} concentration was 14.3 mM. Mn^{2+} could partially substitute for Mg^{2+} , but Cu^{2+} inhibited the reaction. The optimum incubation temperature was 25°C, at which temperature the product increased almost linearly up to 3 h and then quickly decreased. Enzyme kinetic studies yielded a K_m value of 3.3 mM with V_{max} of 54 pmol of UMP incorporated/40 min per mg of protein.

The reaction product, labelled with [³H]UTP, was subjected to alkaline hydrolysis and fractionated on a Dowex-1 (formate form) column. There was no radioactivity in the region corresponding to uridine tetraphosphate, indicating that the reaction does not represent the initiation of new RNA chains. Table 2 indicates that most of the radioactivity was recovered in the uridine and 2':3'-UMP peaks, suggesting the addition of UMP to existing RNA molecules. The radioactivity ratio of 4–5.6 in 2':3'-UMP to uridine

Table 1. Requirements for the enzymic reaction

Reaction mixture	UMP incorporated (pmol/40 min mg of protein)	Activity (%)
Complete	323	100
– Cysteine (10 µmol)	82	25
– Cysteine + dithiothreitol (5 µmol)	320	99
– Cysteine + mercaptoethanol (5 µmol)	174	54
– Mg^{2+} (10 µmol)	46	14
+ ATP (150 µmol)	297	92
+ CTP (150 µmol)	310	96
+ GTP (150 µmol)	301	93
+ TTP (150 µmol)	268	83
– [³ H]UTP		
+ [³ H]UDP	7	2
+ [³ H]UMP	7	2
+ [³ H]ATP	13	4
+ [³ H]CTP	38	12
+ [³ H]GTP	72	22
+ [³ H]TTP	16	5
+ Sodium pyrophosphate (5 µmol)	106	22

Table 2. *Characterization of reaction product by alkaline hydrolysis*

A 5-fold assay mixture was employed, with free ribosomes and [³H]UTP. After incubation at 25°C, the reaction was stopped by the addition of sodium pyrophosphate and trichloroacetic acid as described in the Materials and Methods section. The pellet resulting from centrifugation at 20000g for 10 min was washed twice with 10 ml of cold 5% trichloroacetic acid. The final pellet was suspended in 2.0 ml of cold water and hydrolysed with an equal volume of 0.6M-KOH for 18 h at 37°C. The hydrolysed sample was neutralized (pH 7.0) before the addition of 0.4 mg each of non-radioactive uridine, 2':3'-UMP, 5'-UDP and 5'-UTP to serve as markers. The solution was placed on a Dowex 1 (formate form; 200–400 mesh) column (1 cm × 10 cm) and eluted by linear gradients with formic acid and ammonium formate essentially as described by Hurlbert *et al.* (1954). Fractions (5.0 ml) were collected and absorbancy values of 280 nm were determined. The radioactivities of the peak fractions were measured by placing 0.1 ml of the fraction in 10 ml of Bray's solution and by using a liquid-scintillation counter. Similar studies were performed with 75 μCi of [α-³²P]UTP and 23 μCi of [γ-³²P]-UTP.

Labelled UTP	Incubation period (min)	Radioactivity (c.p.m.)			% of total radioactivity in B and C $\left(\frac{B+C}{A} \times 100\right)$	Ratio C/B
		Total input loaded in column (A)	In uridine peak (B)	In 2':3'-UMP peak (C)		
[³ H]UTP	20	12525	2405	7255	77	3.0
	40	22451	3335	18740*	98	5.6
	90	56233	9925	39685	88	4.0
	120	42234	7395	32470	94	4.4
[α- ³² P]UTP	40	29810	0	21420†	80	—
[γ- ³² P]UTP	40	7306	0	0	0	—

* 244 pmol incorporated per mg protein in 40 min.

† 197 pmol incorporated per mg protein in 40 min.

further suggests the synthesis of oligo(U) chains of 5–7-unit lengths. Only 2–6% of the radioactivity was found in the UTP peak. There was no measurable radioactivity in either uridine or 2':3'-UMP peaks with [γ-³²P]UTP, but with [α-³²P]UTP radioactivity was recovered only in the 2':3'-UMP peak.

The UMP was apparently added to existing RNA molecules. When the RNA was extracted with cold phenol at the end of the reaction and fractionated on sucrose gradients, radioactivity was recovered throughout the gradient. The distribution of radioactivity was exceedingly variable, however, perhaps as a result of degradation of the RNA. Nonetheless, the results clearly indicate that the homopolymerase reaction resulted in the addition of UMP to existing RNA molecules.

A comparison of the various ribosomal preparations, listed in Table 3, clearly indicated that the highest UMP incorporation was observed in the free ribosomal preparation whether the radioactivity was determined per mg of protein or per mg of RNA. The membrane-bound ribosomes showed little incorporation, although an increase was observed when bound ribosomes were liberated with deoxycholate or with salt and puromycin treatment. A lower incorporation was observed after treatment of free ribosomes with deoxycholate. Microsomal fractions, representing a mixture of free and membrane-bound ribosomes, displayed relatively poor polymerase activity. Analysis of various cellular fractions dis-

Table 3. *Enzymic analyses of various ribosomal preparations and cellular fractions*

Cellular fraction	UMP incorporation (pmol/40 min per mg of protein)	Activity (%)
Free ribosomes	294	100
Free ribosomes + deoxycholate treatment	103	35
Bound ribosomes	9	3
Bound ribosomes + deoxycholate treatment	41	14
Bound ribosomes liberated with salt and puromycin	18	6
Microsomal fraction	65	22
Nuclei	43	15
Mitochondria	25	8
Post-mitochondrial supernatant*	24	8

* Precipitated with (NH₄)₂SO₄ and dissolved in Tris buffer.

played minimal incorporation in the nuclear, mitochondrial and post-mitochondrial supernatant preparations.

Poly(U) polymerase in partially purified enzyme

There was only a 20% loss of enzymic activity as

Table 4. *Recovery of enzymic activity during purification of poly(U) polymerase*

The results represent averages of two separate experiments, each utilizing 12 ribosomal pellets. Samples were removed from various stages of purification and analysed for poly(U) polymerase activity as described in the text.

Preparation	Enzyme activity		Total protein (mg)	Specific radioactivity (total c.p.m./total mg)	Activity (%)
	(c.p.m./pellet)	(total c.p.m.)			
Free ribosomal protein preparation after sonification and dialysis	27754	333038	15.28	21799	100
Enzyme preparation after 30–45%-(NH ₄) ₂ SO ₄ precipitation	24633	295595	5.99	49348	89
Enzyme preparation after protamine sulphate, 50%-(NH ₄) ₂ SO ₄ precipitation and dialysis	22388	268650	4.91	54715	81

Fig. 1. *Sephadex G-150 column chromatography of the enzyme preparation*

The protein solution prepared from free ribosomes was placed on a Sephadex G-150 column, 30cm $\times$ 3 cm, equilibrated with 0.05M-(NH₄)₂SO₄ in buffer B, pH 7.4. Fractions (100 drops, 3.0ml) were collected and absorbance at 280nm was recorded. Fractions were analysed for poly(U) polymerase activity. Fraction A represents the pooled fractions nos. 11–16. The standard assay for poly(U) polymerase activity included 50 μ mol of Tris buffer, pH 8.0, 4 μ mol of MgCl₂, 10 μ mol of dithiothreitol, 15 μ Ci of [³H]UTP (specific radioactivity, 100 μ Ci/ μ mol) and 0.10ml of enzyme solution in a final volume of 0.7ml. The enzyme was assayed at 10–30 μ g of protein and 0.1–0.2 μ g of RNA per assay. The incubation temperature was 32°C. In the assay with exogenous RNA, 75 μ g of cold-phenol-extracted ribosomal RNA was added to the reaction mixture (Kidson *et al.*, 1963). The assay was run for 40 min at 32°C and terminated by adding sodium pyrophosphate and trichloroacetic acid as described for free ribosomal preparation, and radioactivity was similarly determined. ○—○, Reaction with endogenous primer; ●—●, reaction with 75 μ g of ribosomal RNA; ●····●, A₂₈₀.

a result of purification procedures before column chromatography (Table 4).

With Sephadex G-150 column chromatography the

sole peak of poly(U) polymerase activity utilizing endogenous RNA primer (fraction A) was coincident with the absorbance peak at 280 nm (Fig. 1). Fraction

Table 5. *Characterization of reaction product by alkaline hydrolysis*

A 3-fold assay mixture was employed, with fraction A. The reaction was stopped by the addition of sodium pyrophosphate and trichloroacetic acid as described in the Materials and Methods section. The final pellet was suspended in 2.0ml of cold water and hydrolysed in 0.3M-KOH for 18h at 37°C. The hydrolysed sample was neutralized and fractionated by Dowex 1 (formate form) column chromatography as described in Table 2. Expt. 1, fraction 10; Expt. 2, pooled samples of fraction A; Expt. 3, 225 µg of ribosomal RNA added with pooled fraction A.

Expt. no.	Radioactivity (c.p.m.)			% of total radioactivity in B and C $\left(\frac{B+C}{A} \times 100\right)$	Ratio C/B
	Total input loaded in column (A)	In uridine peak (B)	In 2':3'-UMP peak (C)		
1	36360	1931	31216	91	16.2
2	28864	1689	23477	87	13.9
3	1920	536	343	46	0.64

Table 6. *Effects of reagents on fraction A activity*

Reaction mixture	Activity (%)
Complete	100
- Mg ²⁺	31
- Mg ²⁺ + Mn ²⁺ (0.1 µmol)	34
- [³ H]UTP + [³ H]ATP	25
+ [³ H]CTP	19
+ [³ H]GTP	18
+ [³ H]UDP	10
+ [³ H]UMP	24
+ RNA (phenol-extracted rRNA) (75 µg)	78
+ ATP, GTP, CTP (0.3 µmol of each)	105
+ Poly(U) (100 µmol)	75
+ Poly(A) (100 µmol)	66
+ ATP-generating system (creatine phosphate)	120
+ Actinomycin D (1.25 µg)	100
+ Deoxyribonuclease (1 µg)	100

A had a specific activity of 3168 pmol/mg of protein; its activity increased linearly for 2h, and the enzyme was stable for 3–4 days at 4°C. The reaction product was analysed after alkaline hydrolysis; the peak fraction of fraction A exhibited a 2':3'-UMP/uridine ratio of 16.2 (Table 5), and for the pooled fraction A the ratio was 13.9. When exogenous ribosomal RNA was added to fraction A assay, not only was there a marked inhibition of enzymic activity, but the 2'-3'-UMP/uridine ratio was lowered to 0.64.

Sucrose-gradient analysis of the reaction product from fraction A after 10, 20 and 40 min of incubation demonstrated only an increasing amount of radioactivity with time in the low-molecular-weight zone without any specific pattern of radioactivity peaks.

The effects of various reagents on fraction A activity are listed in Table 6. The results indicated that the enzyme was a ribohomopolymerase involving poly(U) with specific requirements for endogenous RNA and Mg²⁺. The addition of exogenous ribo-

somal RNA, poly(U) or poly(A) actually depressed the enzymic activity.

Fraction A was placed on a CM-cellulose column for further fractionation. The results (Fig. 2) indicated the presence of several forms of poly(U) polymerase demonstrating different primer requirements for either exogenous or endogenous RNA. A few of the endogenous RNA-requiring poly(U) polymerase activities were coincident with the peak activities of the exogenous RNA-requiring enzymes; however, others were clearly separate.

Alkaline hydrolysis of the reaction product from some of the CM-cellulose peaks displayed relatively low 2',3'-UMP/uridine ratios (Table 7), especially in the reaction with exogenous ribosomal RNA. Longer chain lengths of UMP were observed in reactions with endogenous RNA primer.

Sucrose-gradient analyses of the cold-phenol-extracted RNA of the reaction product obtained after short incubations displayed radioactivity in the 28S and 18S peaks of exogenous ribosomal RNA; after longer incubations (at 32°C) there was extensive degradation of 28S and 18S ribosomal RNA. Sucrose-gradient analyses of the cold-phenol-extracted RNA of the reaction product from the endogenous primer reaction showed a radioactivity peak in the low-molecular-weight zone. The fractions containing radioactivity were pooled and placed in a 100°C water bath for 5 min; the RNA was extracted with cold phenol and placed on a second sucrose gradient. Analyses demonstrated the same radioactivity peak in the low-molecular-weight zone. These findings suggest that the short poly(U) chains were linked to pre-existing RNA species with linkage more specific than hydrogen-bonding.

Although components of fraction A could be separated by DEAE-cellulose chromatography, the results were not as reproducible nor as sharply delineated as those obtained with CM-cellulose. With both columns, however, the resulting poly(U) poly-

Fig. 2. *CM-cellulose chromatography*

Fraction A samples were pooled and the protein was precipitated with the addition of an equal volume of 100%-satd. $(\text{NH}_4)_2\text{SO}_4$. The pellet was suspended in 0.05 M- $(\text{NH}_4)_2\text{SO}_4$ with buffer C (0.01 M-phosphate/5 mM- MgCl_2 /0.1 mM-dithiothreitol, pH 6.0). The protein solution was placed on a CM-cellulose column (10 cm $\times$ 1 cm) equilibrated with 0.05 M- $(\text{NH}_4)_2\text{SO}_4$ in buffer C, pH 6.0, and eluted with 0.1 M- $(\text{NH}_4)_2\text{SO}_4$ in buffer C, pH 6.0. Fractions (30 drops, 1.0 ml) were collected and immediately analysed for enzymic activity. The assay system was the same as that described in Fig. 1, except 10 μmol of MgCl_2 was utilized in the reaction mixture. $\circ\text{---}\circ$, Reaction with endogenous primer; $\bullet\text{---}\bullet$, reaction with 75 μg of ribosomal RNA; $\bullet\cdots\bullet$, A_{280} .

Table 7. *Characterization of reaction product by alkaline hydrolysis*

The product from 3-fold assay of CM-cellulose fractions (Fig. 2) was hydrolysed and analysed by Dowex 1 (formate form) chromatography as described in Table 2.

CM-cellulose fraction no.	Radioactivity (c.p.m.)			% of total radioactivity in B and C $\left(\frac{B+C}{A} \times 100\right)$	Ratio C/B
	Total input loaded in column (A)	In uridine peak (B)	In 2'-3'-UMP peak (C)		
Without exogenous RNA					
6	4953	895	3343	86	3.7
8	12352	2090	2883	40	1.4
14	2332	750	430	51	0.6
With exogenous ribosomal RNA					
11	2024	483	447	46	0.9
12	1962	512	268	40	0.5
15	1733	502	223	42	0.4

merase activities were lost in 45–60 min. Because of the inability to stabilize the enzyme, our attempts to isolate and characterize the activity with the endogenous primer were unsuccessful.

Discussion

The present results indicate that the specific requirements for poly(U) polymerase activity depend upon the degree of purification of the enzyme preparation. Our initial report on poly(U) polymerase activity in the free ribosomes of the rat liver utilized a crude preparation that did not require exogenous RNA for maximal reaction (Hayashi, 1975). During the period when our studies with a free ribosomal preparation had been completed, Milchev & Hadjiolov (1978) reported the coexistence of poly(U) polymerase and poly(A) polymerase in the cytoplasmic ribosomes of Ehrlich ascites tumour cells in a similar ribosomal preparation without exogenous RNA. Wilkie & Smellie (1968*b*) had reported the presence of poly(U) polymerase in the free ribosomes of rat liver, utilizing an unpurified ribosomal preparation that required exogenous polymerized yeast RNA for enzymic activity.

The RNA primer requirements of poly(U) polymerase among eukaryotic systems have been reported to vary widely. Poly(U) polymerase prepared from microsomal fractions and from free ribosomes of rat liver definitely required either yeast RNA (Wilkie & Smellie, 1968*a,b*) or ribosomal RNA (Klemperer, 1963); the enzyme from microsomal and cytoplasmic fractions of Landschütz ascites-tumour cells and Ehrlich ascites-carcinoma cells required exogenous RNA extracted from its own tumour cells (Wykes & Smellie, 1966; Burdon & Smellie, 1961). The microsomal fraction from rat brain demonstrated poly(U) polymerase activity that preferentially utilized 28 S and 18 S ribosomal RNA species extracted from either rat brain or rat liver (Hozumi *et al.*, 1975). The enzyme in tobacco leaves exhibited an even more specific primer requirement for poly(A) (Brishamar & Juntti, 1975). The only poly(U) polymerases that do not require exogenous RNA were found in the cytoplasmic extract of BHK₂₁ cells, in cytoplasmic ribosomes of Ehrlich ascites-tumour cells (Villarreal & Holland, 1974) and in the nuclear ribosomes of calf thymus (Burdon, 1963). The present report indicates the presence of several forms of poly(U) polymerase in hepatic free ribosomes with various requirements for endogenous or exogenous primers.

Fraction A separated only by Sephadex G-150 column chromatography differed markedly from the previously reported poly(U) polymerases of eukaryotic systems in demonstrating an unusual dependence upon an endogenous primer. The activity was sharply decreased with exogenous RNA concentration

greater than 10 µg. Although the enzyme prepared from nuclear ribosomes of calf thymus required no RNA primer, it differed from fraction A in that the addition of poly(U) enhanced its enzymic reaction whereas the presence of other trinucleotides decreased its activity (Burdon, 1963). Also, the same enzyme activity was diminished by sonic disruption, in contrast with fraction A, which showed a 2–3-fold increase in enzymic activity after sonication of the free ribosomal suspension (results not shown). The dependence of fraction A upon an endogenous primer even after several purification procedures, including protamine sulphate treatment followed by separation with Sephadex chromatography, suggests that the primer is tightly bound to the enzyme. Furthermore, enzyme A has demonstrated the longest chain lengths of poly(U) compared with those reported previously from eukaryotic systems.

The present data do not specifically indicate that the endogenous primer is RNA. Such detail will require purification of enzyme to homogeneity in addition to a stable enzyme fraction. However, the presence of radioactivity in the sucrose gradient after cold-phenol extraction of the reaction product from both the exogenous- and endogenous-RNA-requiring poly(U) polymerase does suggest that in all probability the primer is RNA.

The poly(U) polymerase activities reported herein have been found only in the free ribosomal preparations from rat liver homogenates. Both the membrane-bound ribosomes and microsomal fraction produced minimal or no activity; the use of deoxycholate in any phase of the preparation strongly inhibited the enzymic reaction, as was found for extracts from BHK₂₁ cells (Villarreal & Holland, 1974). Our inability to demonstrate enzymic activities in these ribosomal preparations may possibly reflect the presence of higher ribonuclease activity in such preparations. Furthermore, the present studies do not rule out the possibility that the poly(U) polymerase activity observed in the free ribosomes may have resulted from an adventitious binding of poly(U) polymerase from other sources such as the nuclei during the homogenization procedure.

All previous reports on poly(U) polymerase have indicated a single form of enzymic activity, as observed in fraction A of the present study. The various forms of poly(U) polymerases observed after further separation with CM-cellulose chromatography may indicate either multiple forms or complexes of poly(U) polymerase with different primers. Nevertheless, specific changes in poly(U) polymerase activity were observed after CM-cellulose separation: there was decreased chain length of reaction product, the enzyme became unstable even though it still retained activity with endogenous primer, and there was less inhibition by exogenous RNA. These

changes may be the result of separation of enzyme from primer, either transient or permanent, or the result of separation of proteins that stabilize the enzyme-primer complex.

The study was supported in part by National Institutes of Health Grant no. HD-04072 and National Institutes of Health Training Grant no. HD-00227. The authors gratefully acknowledge the help and guidance of Dr. Christopher Widnell and Dr. Daisuke Nakada of the University of Pittsburgh School of Medicine.

References

- Blobel, G. & Potter, V. R. (1967a) *J. Mol. Biol.* **26**, 279-292
- Blobel, G. & Potter, V. R. (1967b) *J. Mol. Biol.* **28**, 539-542
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279-285
- Brishammar, S. & Juntti, N. (1975) *Biochim. Biophys. Acta* **383**, 351-358
- Burdon, R. H. (1963) *Biochem. Biophys. Res. Commun.* **11**, 472-476
- Burdon, R. H. & Smellie, R. M. S. (1961) *Biochim. Biophys. Acta* **51**, 153-162
- Darnell, J. E., Wall, R. & Tushinski, R. J. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1321-1325
- Edmonds, M. & Abrams, R. (1960) *J. Biol. Chem.* **235**, 1142-1149
- Edmonds, M., Vaughn, M. H., Jr. & Nakazato, H. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1336-1340
- Fleck, A. & Munro, H. N. (1962) *Biochim. Biophys. Acta* **55**, 571-583
- Hayashi, T. T. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 503
- Hayashi, T. T. & Kazmierowski, D. (1972) *Biochemistry* **11**, 2371-2378
- Hozumi, N., Haruna, I., Watanabe, I., Mikoshiba, K. & Tsukada, Y. (1975) *Nature (London)* **256**, 337-339
- Hurlbert, R. B., Schmitz, H., Brum, A. F. & Potter, V. R. (1954) *J. Biol. Chem.* **209**, 23-39
- Kidson, C., Kirby, K. S. & Ralph, R. K. (1963) *J. Mol. Biol.* **7**, 312-315
- Klemperer, H. G. (1963) *Biochim. Biophys. Acta* **72**, 403-415
- Korwek, E. L., Nakazato, H., Venkatesan, J. & Edmonds, M. (1976) *Biochemistry* **15**, 4643-4649
- Lee, S. Y., Mendecki, J. & Brawerman, G. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1331-1335
- Lim, L. & Canellakis, E. S. (1970) *Nature (London)* **227**, 710-712
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Milchev, G. I. & Hadjiolov, A. A. (1978) *Eur. J. Biochem.* **84**, 113-121
- Molloy, G. R., Jelinek, W., Saldit, M. & Darnell, J. E. (1974) *Cell* **1**, 43-53
- Roeder, R. G. & Rutter, W. J. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **65**, 675-682
- Villarreal, L. P. & Holland, J. J. (1974) *J. Virol.* **14**, 441-445
- Wilkie, N. M. & Smellie, R. M. S. (1968a) *Biochem. J.* **109**, 229-238
- Wilkie, N. M. & Smellie, R. M. S. (1968b) *Biochem. J.* **109**, 485-494
- Wykes, J. R. & Smellie, R. M. S. (1966) *Biochem. J.* **99**, 347-355