Deoxyribonucleic Acid-Dependent Ribonucleic Acid Polymerases from Normal and Polyoma-Transformed BHK-21/C13 Cells

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DNA-dependent RNA polymerase (EC 2.7.7.6) activities from normal BHK-21/C13 cells and from BHK-21/C13 cells transformed by polyoma virus (PYY cells) were solubilized and fractionated on columns of DEAE-Sephadex. Various properties of the A and B enzymes from the two types of cell were compared. 1. The yields of polymerase relative to the DNA content of the nuclear preparations are similar for both cell types. 2. The ionic-strength optima of polymerases A and B are 12.5 mM and 100 mM with respect to $(\text{NH}_4)_2\text{SO}_4$ for both cell types. 3. The $\text{Mn}^{2+}/\text{Mg}^{2+}$ activity ratio (measured at the respective optimum for each cation) for polymerase A from BHK-21/C13 cells was 1.48 and for the polymerase A from PYY cells was 0.55. The corresponding ratios for polymerase B were 10.11 for BHK-21/C13 cells and 22.75 for PYY cells. 4. Minor differences in the ability of the A polymerases to transcribe native and denatured DNA templates were observed; such differences were not apparent when the B polymerases were compared. 5. All the polymerases were inhibited completely by actinomycin D and by rifampicin AF/013, but not markedly so by rifampicin. α -Amanitin inhibited polymerase B but not polymerase A.

Multiple DNA-dependent RNA polymerases (EC 2.7.7.6) have been detected in several eukaryotic cell types (Blatti *et al.*, 1970; Chambon *et al.*, 1970; Chesterton & Butterworth, 1971*a,b*). Five different forms of the enzyme have been reported. They are termed AI, AII, AIII, BI and BII, the activities of the form A enzymes being resistant to the inhibitory action of the fungal toxin α -amanitin, whereas the activities of the B polymerases are sensitive to this compound (Kedinger *et al.*, 1971). Roeder & Rutter (1970) have shown that forms AI and AII are located in the nucleolus, whereas the B forms are found in the nucleoplasm. There is evidence that polymerase AIII has a mitochondrial location (Horgen & Griffin, 1971). The A and B enzymes have different ionic-strength optima and bivalent-cation preferences (Chesterton & Butterworth, 1971*a*; Roeder & Rutter, 1970).

Work by Tocchini-Valentini & Crippa (1970) using oocytes of *Xenopus laevis* has indicated that nucleolar ribosomal DNA appears to bind polymerase AI but not the B polymerases, suggesting that polymerase AI is concerned specifically with the synthesis of ribosomal RNA. Butterworth *et al.* (1971) have found that rat liver chromatin is transcribed by the B polymerases but not by the AI or AII enzymes; this observation indicates that nucleoplasmic DNA is transcribed solely by the B enzymes. Further, transcription of the euchromatin regions of interphase chromosomes is initiated by the form-B enzyme, not

in condensed chromatin but within the euchromatin stretches (Chesterton *et al.*, 1974). The apparent difference in gene specificity of the enzymes is supported by Zylber & Penman (1971), who provided evidence that in intact HeLa-cell nuclei the nucleolar polymerases synthesized precursor ribosomal RNA and the nucleoplasmic enzymes synthesized heterogeneous nuclear RNA. Thus it can be postulated that the multiple RNA polymerases of eukaryotic cells control, at least in part, the nature and activities of such cells. This hypothesis was tested by Chesterton *et al.* (1972), who showed that a minimal-deviation rat hepatoma cell line contained a substantially higher amount of polymerase AI than did fully differentiated rat liver cells.

In view of these findings it is important to determine whether such a difference is peculiar to the rat liver system or whether it applies also to other cell types. Hence we have undertaken an examination of the RNA polymerase activities extracted from BHK-21/C13 cells transformed by polyoma virus to determine whether the enzyme content and properties differ. The project has also enabled us to characterize the RNA polymerase activities of a cell system which is widely used for the studies of virus growth and of the cell cycle.

Experimental

Materials

Calf thymus DNA (type I), ATP, RNAase† A, RNAase-free DNAase, $(\text{NH}_4)_2\text{SO}_4$ (grade 1 purified)

† Abbreviations: RNAase, ribonuclease; DNAase, deoxyribonuclease.

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and Trizma base (reagent grade) were purchased from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey KT2 7BH, U.K. CTP, GTP and UTP were obtained from P-L Biochemicals Inc., Milwaukee, Wis., U.S.A.; [$5\text{-}^3\text{H}$]UTP (2Ci/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K.; α -amanitin was from Boehringer-Ingelheim, Isleworth, Middx, U.K.; actinomycin D was from Boehringer Corp (London) Ltd., London W5 2TZ, U.K.; and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 2,5-diphenyloxazole and 1-thioglycerol were from Koch-Light Laboratories, Colnbrook, Bucks., U.K. All other chemicals were of AnalaR grade and were purchased from BDH Chemicals, Poole, Dorset, U.K. Whatman DE-81 paper discs were obtained from Reeve Angel Scientific Ltd., London SE1 6BD, U.K., and DEAE-Sephadex A-25 was from Pharmacia (G.B.) Ltd., London W5 5SS, U.K.

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Cells

Baby-hamster kidney cells (BHK-21/C13; Macpherson & Stoker, 1962) and BHK-21/C13 cells transformed by polyoma virus (PYY cells) were obtained from Flow Laboratories Ltd., Irvine, Ayrshire, U.K. Both types of cell were cultured as described by Rolton & Keir (1974).

Cell disruption and enzyme extraction

BHK-21/C13 and PYY cells were processed in exactly the same way except where indicated. Confluent cells from 20 bottles were removed from the glass with 0.5mM-EDTA at 37°C. All subsequent operations were conducted at 4°C. The cells (4×10^9) were washed once in iso-osmotic buffer (200ml; 0.05M-thioglycerol, 0.32M-sucrose, 0.01M-Tris-HCl, pH7.4), resuspended and allowed to swell in hypo-osmotic buffer (0.05M-thioglycerol, 0.01M-Tris-HCl, pH7.4) at a concentration of 2×10^7 cells/ml. After swelling for 5min (BHK-21/C13 cells) or 10min (PYY cells), the cells were disrupted in a glass homogenizer with two strokes of a Teflon pestle (clearance 0.075mm), by using a Tri-R Stir-R homogenizer at 1100rev./min. These conditions gave minimal nuclear damage with maximal cell breakage. The nuclei were collected by centrifugation in the MSE 2L Mistral centrifuge (10min at 4°C, 100g, r_{av} , 17cm) and resuspended at a concentration of about 10^8 nuclei/ml in the iso-osmotic buffer.

DNA-dependent RNA polymerase activities were extracted from the nuclei by a technique similar to that of Roeder & Rutter (1970). The nuclear suspension was made 0.3M with respect to $(\text{NH}_4)_2\text{SO}_4$, and the nuclei were disrupted by sonication with a Dawe

Soniprobe at maximum power for 2min in 20s bursts with intermittent cooling of the preparation in ice. After an immediate 3-fold dilution into 25% glycerol buffer [0.05M-thioglycerol, 5mM-MgCl₂, 0.5mM-EDTA, 25% (v/v) glycerol, 0.05M-Tris-HCl, pH8.0], the disrupted nuclei were centrifuged at 4°C for 1h at 100000g in the 8×25 ml fixed-angle rotor (no. 59594; r_{av} , 6.89cm) of the MSE Superspeed 65 ultracentrifuge. The pellet was discarded and the supernatant fluid adjusted to near-saturation by the addition of 0.42g of $(\text{NH}_4)_2\text{SO}_4$ /ml over a period of 1h with very gentle stirring. The precipitate was sedimented at 4°C for 30min at 75400g in the 8×50 ml fixed-angle rotor (no. 59584; r_{max} , 10.8cm) of the MSE High-Speed 25 centrifuge and redissolved in a small volume of 30% glycerol buffer [0.05M-thioglycerol, 5mM-MgCl₂, 0.5mM-EDTA, 30% (v/v) glycerol, 0.05M-Tris-HCl, pH8.0] containing 0.05M- $(\text{NH}_4)_2\text{SO}_4$. This concentrated solution was dialysed overnight against 5 litres of the 30% glycerol buffer containing 0.05M- $(\text{NH}_4)_2\text{SO}_4$, and any remaining insoluble material was removed by centrifugation at 4°C for 1h at 130000g in the 3×5 ml swing-out rotor (no. 59589; r_{av} , 7.38cm) of the MSE Superspeed 65 ultracentrifuge. The supernatant fraction was either stored at -70°C or immediately applied to a column (2cm $\times$ 30cm) of DEAE-Sephadex A-25 previously equilibrated with the 30% glycerol buffer containing 0.05M- $(\text{NH}_4)_2\text{SO}_4$. After loading, the column was washed with the same buffer, and then with the 30% glycerol buffer containing 0.1M- $(\text{NH}_4)_2\text{SO}_4$ until no further protein was eluted. The RNA polymerase activities were eluted from the column with a linear gradient of 0.1–0.4M- $(\text{NH}_4)_2\text{SO}_4$ in the 30% glycerol buffer. Fractions containing enzyme were dialysed for 8h against two 2-litre changes of the 30% glycerol buffer, and were finally stored at -70°C.

Assay of DNA-dependent RNA polymerases

The assay mixture (0.2ml) contained: ATP, CTP and GTP (0.6mM each), [$5\text{-}^3\text{H}$]UTP (0.1mM, 50Ci/ μ mol), Tris-HCl buffer, pH8.0 at 37°C (56mM), thioglycerol (5mM) and enzyme solution (50 μ l, 10–100 μ g of protein). Bivalent-cation concentration, ionic strength and the DNA template concentration were as stated in the Results section. The assay mixtures were incubated for 3min at 37°C and the reaction was stopped by spotting 0.1ml on to discs of Whatman DE81 paper (2.3cm diam.). The discs were allowed to dry at room temperature and then washed six times for 5min each with 5% (w/v) Na₂HPO₄, twice with water, twice with ethanol and once with diethyl ether. After drying in an oven at 60°C, the discs were transferred to scintillation vials and measured for radioactivity in the Tracerlab Corumatic 100 liquid-scintillation spectrometer (efficiency of counting 3.3%) with the scintillation

fluid (10ml/vial) described by Hayton *et al.* (1973). One unit of polymerase activity catalyses the incorporation of 1pmol of [^3H]UMP residues/min at 37°C into the RNA product.

Assay of DNAase

(a) The assay mixture (0.25ml) contained 3 μg of ^3H -labelled DNA (10^3 c.p.m.) from *Escherichia coli*; Mg^{2+} (4mM); sodium acetate buffer, pH 5.9 (50mM); bovine serum albumin (0.02%, w/v); 2-mercaptoethanol (8mM); and 0.05ml of enzyme. After 30min at 37°C the samples were chilled on ice to 0°C and 0.1ml of calf thymus DNA (2.5mg/ml) was added followed by 0.25ml of 7% (v/v) HClO_4 . After 20min at 0°C the samples were centrifuged ($1000g_{\text{av}}$, 45min, 2°C). The acid-soluble radioactivity in the resulting supernatant was measured for radioactivity as described above by transfer of 0.1ml portions to vials containing 10ml of the scintillation fluid made 33% (v/v) with Triton X-100. The assay was performed also at pH 8.5.

(b) Similar assays at pH 8.0 followed the conversion of covalently closed circular DNA from bacteriophage λ from a rapidly sedimenting molecular species into slowly sedimenting single-stranded circular and linear molecules, by centrifugation through alkaline sucrose density gradients. The centrifugation details and the preparation of ^3H -labelled covalently closed circular λ DNA by the action of polynucleotide ligase from *E. coli* on Hershey circles of λ DNA were as described by Feldberg *et al.* (1972).

Preparation of DNA from BHK-21/C13 cells

DNA from BHK-21/C13 cells was prepared from 20 roller bottles of confluent cells by extraction overnight at 37°C with 10ml of 2% (w/v) sodium dodecyl sulphate–0.15M-NaCl–15mM-trisodium citrate–0.1M-EDTA (pH 7.0)/2.24 l bottle. The viscous preparation was extracted twice with water-saturated phenol containing 0.1% (w/v) 8-hydroxyquinoline hemisulphate, each extraction being carried out for 1h at 20°C in an orbital incubator set to give very gentle shaking. The aqueous phase was removed by careful decantation and treated with an equal volume of 2-ethoxyethanol. After gentle mixing, the DNA was allowed to settle and the supernatant was removed by decantation. After two washes in 75% (v/v) ethanol by using the same procedure to remove the supernatant, the DNA was dissolved in 100ml of 15mM-NaCl–1.5mM-trisodium citrate, pH 7.0, overnight at 4°C. The DNA solution was treated with RNAase A (50 $\mu\text{g}/\text{ml}$; previously boiled for 15min in 0.1M-NaCl to destroy any contaminating DNAase) for 30min at 37°C and then made 0.5M with respect to sodium acetate. After a further phenol extraction, the DNA was precipitated with 2-ethoxyethanol, washed twice

with 70% ethanol and dissolved in 5ml of 15mM-NaCl–1.5mM-trisodium citrate, pH 7.0, overnight at 4°C. The DNA solution was adjusted to 500 $\mu\text{g}/\text{ml}$ and stored at –20°C. Such DNA preparations had an extinction ratio (260nm:280nm) of 1.9. Thermal denaturation of calf thymus and BHK-21/C13-cell DNA was carried out by heating these standard solutions for 10min at 100°C followed by rapid cool-in in ice.

Other methods

DNA was determined by the method of Burton (1956). Protein was determined, with bovine serum albumin as standard, by the method of Lowry *et al.* (1951), after samples had been freed from interfering thioglycerol by the procedure of Bennet (1967).

Results

RNA polymerase: solubilization and fractionation

Typical extraction and purification of the RNA polymerase activities from the two types of cell (Table 1) gave an apparent increase in specific activity of 20- to 50-fold. It is recognized that the recoveries presented in Table 1 are unlikely to be precise, since the template presumably changes during purification from endogenous native DNA in the nuclei (to which is added denatured DNA in the assay), to the added denatured DNA only in the case of the purified fractions. Moreover, the different enzymes differ in their preferences between these two types of template; this will be described below (Fig. 4). Allowing for the preference of the RNA polymerases for a denatured template, the specific activities obtained for the enzymes from BHK-21/C13 cells and from PYY cells are similar to those reported for the enzymes from calf thymus tissue (Chambon *et al.*, 1970), from KB cells (Keller & Goor, 1970) and from *Xenopus laevis* (Roeder *et al.*, 1970); all these authors used native DNA as template. The elution profile (Fig. 1) of a preparation of the BHK-21/C13 enzymes revealed a pattern identical with that of preparations from the PYY cells. The B polymerases from both types of cell were completely inhibited by α -amanitin, whereas the A enzymes were unaffected by the toxin. Therefore by comparison with the findings of other workers (Roeder & Rutter, 1970; Chesterton & Butterworth, 1971b) it would appear that the A and B enzymes from BHK-21/C13 and PYY cells are respectively nucleolar and nucleoplasmic in origin.

We have not yet perfected techniques which satisfactorily separate the A enzymes into AI and AII or the B enzymes into BI and BII, for either of the cell systems used. A major difficulty in this respect stems from the rapid and irreversible loss of enzyme activity in the absence of MgCl_2 , thus precluding the

Table 1. *Fractionation of DNA polymerases from BHK-21/C13 and PYY cells*

Nuclei were prepared from 20 bottles of confluent cells. In addition to the basic constituents described in the Experimental section, the assays contained 100 mM-(NH₄)₂SO₄, 10 µg of heat-denatured calf thymus DNA and 1.6 mM-MnCl₂, except for the pooled fractions of enzyme A from the DEAE-Sephadex column where the (NH₄)₂SO₄ concentration was lowered to 12.5 mM.

Fraction	BHK-21/C13 cells			PYY cells		
	Enzyme activity (units)	Recovery (%)	Specific activity (units/mg of protein)	Enzyme activity (units)	Recovery (%)	Specific activity (units/mg of protein)
Nuclei	36000	100	219	63000	100	243
100000g supernatant	22800	63	200	45000	71	288
Dialysed concentrated (NH ₄) ₂ SO ₄ supernatant	26460	74	400	47250	75	643
DEAE-Sephadex						
Pooled A	8550	24	4280	6690	11	3470
Pooled B	17370	48	4670	30180	48	12020

Fig. 1. *Chromatography of RNA polymerase from BHK-21/C13 cells on DEAE-Sephadex*

A sample (66 mg of protein in 10 ml) of the concentrated supernatant fluid was applied to a column (2 cm × 30 cm) of DEAE-Sephadex A-25, and washed and eluted as described in the Experimental section. Fractions (2.5 ml) were collected and assayed for polymerase activity with 1.6 mM-MnCl₂ and 10 µg of heat-denatured calf thymus DNA in the presence (○) or absence (●) of α -amanitin at 1 µg/assay. The broken line is a chart-recorder trace of the extinction at 280 nm of the column eluate. The solid line without symbols describes the (NH₄)₂SO₄ concentration gradient passed through the column.

use of chromatography on phosphocellulose, the binding capacity of which is destroyed by Mg²⁺ ions. Attempts at further purification of the B enzymes by using calcium phosphate gel have resulted in unacceptably high losses of enzyme activity.

Assays for DNAase activity by method (a) revealed that less than 0.5% of the DNA was solubilized at either pH 5.0 or pH 8.5. Assays for DNAase by method (b) showed that treatment of the closed circular molecules for 60 min at 37°C with the RNA polymerase fractions did not shift their position relative to that of the untreated controls on alkaline sucrose density gradients. We have concluded from these experiments that our RNA polymerase preparations contain negligible amounts of DNAase activity.

Properties of the RNA polymerase activities

The ionic-strength optima of the A and B polymerases were different (Fig. 2); the responses to increasing concentrations of (NH₄)₂SO₄ were very similar to those of other eukaryotic systems (Roeder & Rutter, 1969; Chambon *et al.*, 1970). In contrast there was no significant difference between the corresponding A polymerases and B polymerases from BHK-21/C13 and PYY cells. However, differences were noted between the two polymerase A activities when the enzymes were assayed over a range of concentrations of Mn²⁺ and Mg²⁺ ions (Fig. 3). The BHK-cell polymerase A gave highest activity with MnCl₂, whereas the PYY-cell polymerase A gave more activity with MgCl₂.

Fig. 2. RNA polymerase activities as a function of ionic strength of the assay mixture

In addition to the standard constituents described in the Experimental section, assay mixtures contained $10\mu\text{g}$ of native DNA from BHK-21/C13 cells, 1.6mM-MnCl_2 and $(\text{NH}_4)_2\text{SO}_4$ as indicated. BHK-21/C13-cell polymerase A (●) 30 units and polymerase B (○) 14 units; PYY-cell polymerase A (▲) 18.6 units and polymerase B (△) 15.1 units.

Fig. 3. Effect of bivalent cations on the RNA polymerase activities

The form-A polymerases were assayed in the presence of $12.5\text{mM-(NH}_4)_2\text{SO}_4$ and the form-B polymerases with $100\text{mM-(NH}_4)_2\text{SO}_4$. All assays contained $10\mu\text{g}$ of native DNA from BHK-21/C13 cells. The bivalent cations and their concentrations were as shown. Those assays containing MnCl_2 also contained 1.25mM-MgCl_2 from the enzyme-containing buffer. ●, Mn^{2+} ; ○, Mg^{2+} .

The template preferences of the four enzymes were also studied (Fig. 4). With the A enzyme from BHK-21/C13 cells, there was a small, but nevertheless clear

and reproducible, preference for a denatured rather than a native DNA template. Such a preference was not apparent with the A polymerase of the PYY cells.

Fig. 4. RNA polymerase activities as a function of DNA template concentration

Assay mixtures for polymerase A contained 12.5 mM-(NH₄)₂SO₄ and those for polymerase B contained 100 mM-(NH₄)₂SO₄. All assays contained 1.6 mM-MnCl₂. □, Heat-denatured DNA from BHK-21/C13 cells; Δ, heat-denatured DNA from calf thymus; ■, native DNA from BHK-21/C13 cells; ▲, native DNA from calf thymus. BHK-21/C13 RNA polymerases A and B, 30 and 14 units respectively; PYY-cell polymerases A and B, 18.6 and 15.1 units respectively.

The B enzymes from both cell types exhibited markedly more activity on denatured than on native DNA.

The effects of certain assay modifications on the

RNA polymerase activities are shown in Table 2. The enzymes required all four ribonucleoside 5'-triphosphates, and a DNA template. The product was

Table 2. Properties of the RNA polymerases of BHK-21/C13 and PYY cell nuclei

The assays for form-A polymerases contained 12.5mm-(NH₄)₂SO₄ and those for the form-B enzymes contained 100mm-(NH₄)₂SO₄. In addition to the standard constituents described in the Experimental section, the 'complete' assay contained 10μg of native DNA from BHK-21/C13 cells, 1.6mm-MnCl₂ and the following amounts of the indicated enzyme: BHK-21/C13 A, 21.4 units, BHK-21/C13 B, 11.2 units, PYY A, 22.5 units, and PYY B, 15.5 units. In the experiment involving the use of DNAase, the assay mixture was submitted to a preliminary incubation for 10min at 37°C with 20μg of the DNAase, before addition of the polymerase for the standard assay. In the experiment involving RNAase, the latter was added after the standard 3 min at 37°C and incubation was then continued for a further 20min at 37°C; these assays were compared with similar incubations containing water instead of the RNAase, in which cases no significant deviation from the 100%-activity response was observed. The values in parentheses represent the amounts of inhibitor or nuclease added to each assay.

Conditions	RNA polymerase activity of enzyme preparations			
	BHK-21/C13 cells		PYY cells	
	A	B	A	B
	(%)	(%)	(%)	(%)
Complete	100	100	100	100
-ATP	0	0	0	1
-CTP	2	0	0	6
-GTP	0	0	2	1
-DNA	0	0	0	0
+DNAase (20 μg)	0	18	0	26
+RNAase (20 μg)	9	41	7	38
+Actinomycin D (1 μg)	0	0	0	3
+Rifampicin (20 μg)	84	94	71	120
+Rifampicin AF/013 (20 μg)	0	0	0	0
+α-Amanitin (1 μg)	118	0	107	0

sensitive to degradation by RNAase. The apparent partial resistance of the product of the B enzymes to the action of RNAase may be attributable to the inhibition of the nuclease by the 100mm-(NH₄)₂SO₄ in the assay mixture. Other experiments, in which the product was treated with RNAase after it had been collected on the DE81 paper discs, showed that the entire radioactive product was rendered acid-soluble. The A enzymes were not affected by α-amanitin, whereas the B enzymes were strongly inhibited by this toxin. All the enzymes were inhibited by actinomycin D and by the rifampicin derivative AF/013, but not by rifampicin itself. No significant differences in response to the drugs were seen between the corresponding enzymes from the two types of cell.

Table 3. Relative activities of the RNA polymerases from nuclei of BHK-21/C13 and PYY cells

In addition to the basic constituents, the assays contained 1.6mm-MnCl₂ and 10μg of native BHK-21/C13-cell DNA. Assays for form-A polymerase also contained 12.5mm-(NH₄)₂SO₄ and those for form-B enzyme contained 100mm-(NH₄)₂SO₄. The mean values of six experiments are shown together with the standard deviations. The values are calculated relative to the DNA content of the initial nuclear preparations. The mean DNA and protein contents of the batches of the two cell types were similar and were 39±8.3mg and 211±56mg respectively.

Cells	RNA polymerase activity (units/mg of DNA)	
	A	B
BHK-21/C13	69 ± 27	73 ± 23
PYY	70 ± 16	122 ± 36
Ratio PYY/BHK-21/C13	1.02	1.67

Relative activity of the RNA polymerases

A comparison of the RNA polymerase activities relative to the DNA content of the initial nuclear preparations is shown in Table 3. In contrast with the observations of Chesterton *et al.* (1972), no substantial differences between the two types of cells were detected. To test the possibility that a selective loss of one or more of the enzymes may have occurred during the extraction procedure, the initial nuclear preparations were assayed in the presence and absence of α-amanitin and at different salt concentrations, to select for either polymerase A or polymerase B activity. The A/B activity ratio was 1.15 in BHK-21/C13-cell nuclei and 0.64 in PYY-cell nuclei; these ratios should be compared with values of 0.95 and 0.57 respectively calculated for the separated enzymes (Table 3). As the respective ratios are not substantially different, it appears that a selective loss of polymerase activity does not occur during extraction.

Mixing experiments were performed in which polymerase A from BHK cells was added to polymerase A from PYY cells, and in which the respective polymerases B were mixed. In all cases, the activities were strictly additive. Therefore it seems reasonable to conclude that the activities extracted were not affected by the presence of inhibitors or activators in the purified enzymes.

Discussion

The findings reported here show clearly that both BHK-21/C13 cells and PYY cells contain at least two distinct RNA polymerase activities. The properties of the enzymes are very similar to those reported for other eukaryotic systems (Roeder & Rutter, 1969;

Chambon *et al.*, 1970), particularly with respect to their differing responses to $(\text{NH}_4)_2\text{SO}_4$, their response to α -amanitin and their $\text{Mn}^{2+}/\text{Mg}^{2+}$ activity ratios. Also the B polymerases from both BHK-21/C13 and PYY cells show a more marked preference for a denatured rather than a native template compared with the A polymerases. One practical point worth noting is the extreme instability of the enzymes in the absence of Mg^{2+} . If Mg^{2+} is omitted from the elution buffer of the DEAE-Sephadex column, the recovery of the A and B activities is about 40 and 5% respectively. Such an instability has not been reported for RNA polymerase from other eukaryotic cells.

We have been unable to detect any new form of RNA polymerase in the nuclei of PYY cells compared with those of BHK-21/C13 cells. The properties of the corresponding enzymes from the two cell types are essentially the same. Differences do exist, however: the $\text{Mn}^{2+}/\text{Mg}^{2+}$ activity ratios, particularly for the A enzymes, where the preference for Mn^{2+} of the BHK-21/C13-cell form-A polymerase is reversed with form A from PYY cells, which shows a slight preference for Mg^{2+} ions. This difference is reinforced by the finding that the form-A polymerase from BHK-21/C13 cells shows a stronger preference for a denatured template relative to a native template than does the form-A enzyme from PYY cells.

We have been unable to detect substantial differences between the amounts of enzyme activity in the two cell types studied. This is in contrast with the observations of Chesterton *et al.* (1972), who found a considerably greater amount of AI polymerase in rat hepatoma cells relative to rat liver. However, as the authors themselves point out, this may reflect differences in the rate of turnover of ribosomal RNA in a slow-growing, differentiated tissue and a rapidly growing cell line. Both BHK-21/C13 cells and PYY cells are relatively undifferentiated and show rapid growth; a large difference in the rate of turnover of ribosomal RNA would not be expected.

It seems unlikely that inhibitors or activators of the RNA polymerases (Stein & Hausen, 1970; Lee & Dahmus, 1973) are exerting any effect in our enzyme preparations. Such factors as we have found are removed in the flow-through fractions during column chromatography on DEAE-Sephadex. Moreover, the mixing experiments were additive and provided no evidence for the presence of such factors.

This work has laid the basis for our investigation of the effect of virus infection on the RNA polymerase activities of BHK-21/C13 cells and of the ability of the enzymes from both cell types to transcribe viral DNA, including that of polyoma virus.

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