Nishimura, S. (1971), *Proc. Nucl. Acid Res. 2*, 542.
Nishimura, S. (1972), *Progr. Nucl. Acid Res. Mol. Biol. 12*, 49.
Ohashi, Z., Harada, F., and Nishimura, S. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 20*, 239.
Ohashi, Z., Saneyoshi, M., Harada, F., Hara, H., and Nishimura, S. (1970), *Biochem. Biophys. Res. Commun. 40*, 866.
Roy, K. L., Bloom, A., and Söll, D. (1971), *Proc. Nucl. Acid Res. 2*, 524.
Saneyoshi, M., and Nishimura, S. (1970), *Biochim. Biophys. Acta 204*, 389.
Saneyoshi, M., and Nishimura, S. (1971), *Biochim. Biophys. Acta 246*, 123.
Schrier, A. A., and Schimmel, P. R. (1972), *Biochemistry 11*, 1582.
Sekiya, T., Takeishi, K., and Ukita, T. (1969), *Biochim. Biophys. Acta 182*, 411.
Söll, D. (1971), *Science 173*, 293.
Söll, D., Cherayil, J. D., and Bock, R. M. (1967), *J. Mol. Biol. 29*, 97.
Squires, C., and Carbon, J. (1971), *Nature (London), New Biol. 233*, 274.
Varricchio, F., and Seno, T. (1973), *Biochem. Biophys. Res. Commun. 51*, 522.
Walker, R. T., and RajBhandary, U. L. (1972), *J. Biol. Chem. 247*, 4879.
Yoshida, M., Takeishi, K., and Ukita, T. (1970), *Biochem. Biophys. Res. Commun. 39*, 852.
Yoshida, M., Takeishi, K., and Ukita, T. (1971), *Biochim. Biophys. Acta 228*, 153.
Zubay, G. (1962), *J. Mol. Biol. 4*, 347.

Transcription of Chromatin. Initial and Terminal Nucleotides of Ribonucleic Acid Synthesized by Calf Thymus and *Escherichia coli* Ribonucleic Acid Polymerases†

Albert A. Keshgegian,* Garo S. Garibian, and John J. Furth

ABSTRACT: Initial and terminal nucleotides of RNA transcribed from chromatin were compared with RNA transcribed from native and denatured DNA by an RNA polymerase obtained from calf thymus and *Escherichia coli* RNA polymerase. Enzymically transcribed RNA was hydrolyzed in alkali and the hydrolysate resolved by electrophoresis into initial nucleoside tetraphosphate, internal nucleoside monophosphate, and terminal nucleoside. With native calf thymus DNA as template, calf thymus RNA polymerase initiates over 90% with ATP and GTP rather than UTP and CTP. With denatured DNA, initiation by GTP relative to ATP increases over twofold and the RNA is one-fourth as long. With calf thymus chromatin as template, initiation using both calf thymus and *E. coli* RNA polymerases is over 95% with ATP and GTP. The amount of initiation by ATP relative to GTP for the two enzymes is significantly different when chromatin is used as template. These results indicate that calf thymus RNA polymerase initiates predominantly with purine ribonucleoside triphosphates and at sites which may differ from those at which *E. coli* RNA polymerase initiates. The results suggest that the structure of RNA polymerase, in addition to chromatin control factors, may determine the sites at which transcription occurs. Total termination values are two- to fourfold higher than total initiation values. Using calf thymus enzyme, termination with adenosine is significantly higher when chromatin, rather than DNA, is the template. Using *E. coli* enzyme, there is a smaller increase in termination with adenosine. These results suggest preferential termination with adenosine when chromatin is used as template or, alternatively, the presence of an enzyme in chromatin which adds adenosine to the 3′-OH end of RNA.

Differentiation is thought to involve the transcription of only a selected set of genes in a given cell. Control factors which govern this selection of genes are only poorly understood. Non-histone proteins (Paul and Gilmour, 1968) or RNA (Bekhor *et al.*, 1969; Huang and Huang, 1969) may interact with histones to derepress specific genes, *i.e.*, to allow RNA polymerase (RNA nucleotidyltransferase, EC 2.7.7.6) to interact with selected segments of the total DNA. This interaction may also depend upon the structure of the RNA polymerase. We have recently observed that an RNA polymerase preparation obtained from calf thymus and *Escherichia coli* RNA polymerase interact differently with calf thymus chromatin, at least in terms of the kinetic parameters of transcription (Keshgegian and Furth, 1972). One of the predictions of such a difference is that mammalian and bacterial polymerases may transcribe at different sites on the chromatin template. If transcription does occur at different sites, the initial nucleotides of the RNA synthesized may differ. We have therefore investigated the initiation of RNA synthesis by calf thymus RNA polymerase, and compared the initiation on a chromatin template with initiation by *E. coli* RNA polymerase.

A preliminary report of this work has appeared (Keshgegian and Garibian, 1973).

† From the Departments of Biochemistry and Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received May 23, 1973.* This investigation was supported by a grant (2-ROI-GM-10390) from the National Institutes of Health, U. S. Public Health Service. A. A. K. was supported by MSTP National Institutes of Health Training Grant 5T05 GM02046. J. J. F. was supported by a Research Career Development award (GM K3-12888) of the National Institutes of Health, U. S. Public Health Service.

Experimental Section

Materials. Calf thymus DNA was purchased from Worthington Biochemical Corp. [γ-^{32}P]ATP was prepared by the method of Glynn and Chappel (1964). [8-^{3}H]ATP, [8-^{3}H]-GTP, [5-^{3}H]UTP, and [5-^{3}H]CTP were purchased from Schwarz/Mann and ICN. Adenosine 5′-tetraphosphate and guanosine 5′-tetraphosphate were obtained from Sigma Chemical Co.

Enzymes. RNA polymerase was obtained from calf thymus as previously described (Furth *et al.*, 1970). The enzyme is more active with Mn^{2+} than with Mg^{2+} as cofactor, and is more active with native than with denatured DNA (Furth and Austin, 1970). The enzyme is inhibited 50% by high (40 μg/ml) levels of α-amanitin (Furth and Austin, 1970). Upon chromatography on DEAE-Sephadex as described by Roeder and Rutter (1969), the bulk of the enzyme elutes at approximately 0.23 M $(NH_4)_2SO_4$ (G. E. Austin and J. J. Furth, unpublished experiments).

E. coli RNA polymerase was obtained as described by Furth and Pizer (1966) followed by chromatography on DEAE-Sephadex. The preparation contained sigma factor.

Units for both enzymes were defined as nmoles of UMP incorporated into RNA per 10 min with a native calf thymus DNA template. The specific activities (units/mg of protein) were 2.2 for calf thymus enzyme and 67 for *E. coli* enzyme. When incubated with [γ-^{32}P]ATP both enzymes converted approximately one-third of the radioactivity into a nonNorit adsorbable form under standard assay conditions.

Chromatin. Calf thymus chromatin was prepared by the method of Paul and Gilmour (1968), slightly modified, and stored in aliquots at −90°. Prior to use the chromatin was sheared in a VirTis homogenizer at 60% of the maximum rate for 1 min at 4°. The sheared chromatin was centrifuged at 10,000*g* for 5 min to remove insoluble material. $A_{260\ nm}/A_{280\ nm}$, $A_{260\ nm}/A_{240\ nm}$, and $A_{320\ nm}/A_{260\ nm}$ ratios were 1.71, 1.50, and 0.038, respectively. The endogenous RNA polymerase activity was less than 0.01 nmol of [^{3}H]UMP incorporated/10 min (in the amounts of chromatin used).

Assay Procedure. The reaction mixture (1.0 ml) contained: 50 mM Tris-maleate buffer (pH 7.7), 1 mM $MnCl_2$, 12 mM $MgCl_2$, 0.2 M KCl, 0.01 mM EDTA, 8 mM dithiothreitol, 40 μM ^{3}H-labeled nucleoside triphosphate (specific activity 1.0–1.2 $\times 10^6$ counts min^{-1} nmol^{-1}) and 320 μM each of the other three nucleoside triphosphates, DNA or chromatin, and enzyme. The amounts of DNA (native or denatured by heating at 100° for 5 min and cooling in ice) and chromatin were (μmol of deoxynucleotides/l.) 130 and 200, respectively. Average values of enzyme used were 0.5 and 1.5 units for calf thymus and *E. coli* polymerases, respectively. With DNA as template, the reaction was performed in quadruplicate for calf thymus enzyme, and duplicate for *E. coli* enzyme to obtain enough product. With chromatin as template, the reactions were in quadruplicate and quintuplicate for calf thymus and *E. coli* enzymes, respectively. The reaction was allowed to proceed for 10 min at 37° and stopped by chilling in ice. RNA was precipitated by addition of 0.4 ml of 7% $HClO_4$ and 2 mg of albumin as carrier. The mixture was centrifuged for 2 min at 10,000*g* and the pellet washed twice with 1% $HClO_4$.

Alkaline Hydrolysis and Electrophoresis. The final pellet was dissolved in 0.5 N NaOH, a small aliquot removed to determine the extent of incorporation of radioactivity, and the remainder incubated 16 hr at 37°. Separate experiments showed that less than 2% of [γ-^{32}P]ATP is converted to a nonNorit adsorbable form under these conditions. The hydrolysates were combined in groups of 2, neutralized with AG50W-X8 resin (hydrogen form), and concentrated by evaporation. Depending on the nucleoside triphosphate used, a variable amount of ^{3}H in the RNA exchanged into a volatile form (presumably through exchange with water) and was lost upon concentration. Average recoveries were (%): ATP, 12; GTP, 34; CTP, 37; UTP, 65.

Aliquots of the concentrated hydrolysates were mixed with unlabeled markers (nucleoside; 2′(3′)-monophosphate; 5′-diphosphate; 5′-triphosphate for ATP, GTP, UTP, CTP, and in addition 5′-tetraphosphate in experiments with ATP and GTP). The mixture was applied to Whatmann 3MM paper and electrophoresed in 0.05 M sodium citrate buffer (pH 5.0), for 90 min at 20 V/cm. Positions of the markers were determined with an ultraviolet lamp.

Determination of Radioactivity. Aliquots of the hydrolysate and of the concentrated hydrolysate were taken for determination of amount of incorporation and final recovery, respectively. These were added to scintillation vials containing two parts 5 g/l. of 2,5-diphenyloxazole and 0.3 g/l. of 1,4-bis[2-(4-methyl-5-phenyloxazole)] in toluene and one part Triton X-100.

To determine the distribution of radioactivity after electrophoresis, 1-cm wide strips were cut and the radioactivity was eluted by shaking for 1 hr in 1 ml of 0.01 N HCl. In some cases the strip width was less than 1 cm. In these cases, observed counts/min were normalized to counts min^{-1} cm^{-1} for data presentation. Scintillation fluid (10 ml) as described above was added. The presence of paper strips in the vials did not affect the efficiency of counting. Average recovery of radioactivity from the paper was (%): ATP, 59; GTP, 79; CTP, 85; UTP, 88.

Results and Discussion

Analysis of the Method. It has been shown that the initial (5′-end) nucleotide of RNA synthesized *in vitro* retains its 5′-phosphate groups (Maitra and Hurwitz, 1965). Thus alkaline hydrolysis liberates the initial nucleotide as a 5′-triphosphonucleoside 2′(3′)-phosphate (pppNp)[1] (Bremer *et al.*, 1965). The tetraphosphate migrates slightly faster than the corresponding nucleoside triphosphate upon electrophoresis, and indicates the amount of initiation (Bremer *et al.*, 1965).

Representative electropherograms are shown in Figures 1–3. In most cases, a separate radioactivity peak was resolved just ahead of the marker triphosphate. This peak comigrated with guanosine 5′-tetraphosphate when [^{3}H]GTP was used in the reaction, and with adenosine 5′-tetraphosphate when [^{3}H]ATP was used. It was therefore regarded as the tetraphosphate (pppNp).

A similar peak was observed when [^{3}H]CTP was used in the reaction mixture. This peak was in approximately the same relative position as the adenosine and guanosine tetraphosphate peaks. It also increased when denatured DNA was used as template (Figure 2). Initiation by UTP and CTP increases with denaturation of DNA when *E. coli* RNA polymerase (Maitra and Hurwitz, 1965) and form B calf thymus polymerase (Chambon *et al.*, 1970) are used. Therefore this peak was considered to be cytidine tetraphosphate (pppCp).

[1] Abbreviations used are: N, nucleoside; Np, nucleoside 2′(3′)-monophosphate; pNp, 5′-phosphonucleoside 2′(3′)-phosphate; ppN, nucleotide 5′-diphosphate; pppN, nucleoside 5′-triphosphate; pppNp, 5′-triphosphonucleoside 2′(3′)-phosphate; ppppN, nucleoside 5′-tetraphosphate. For specific nucleosides and their derivatives, the appropriate one-letter symbol is substituted for N.

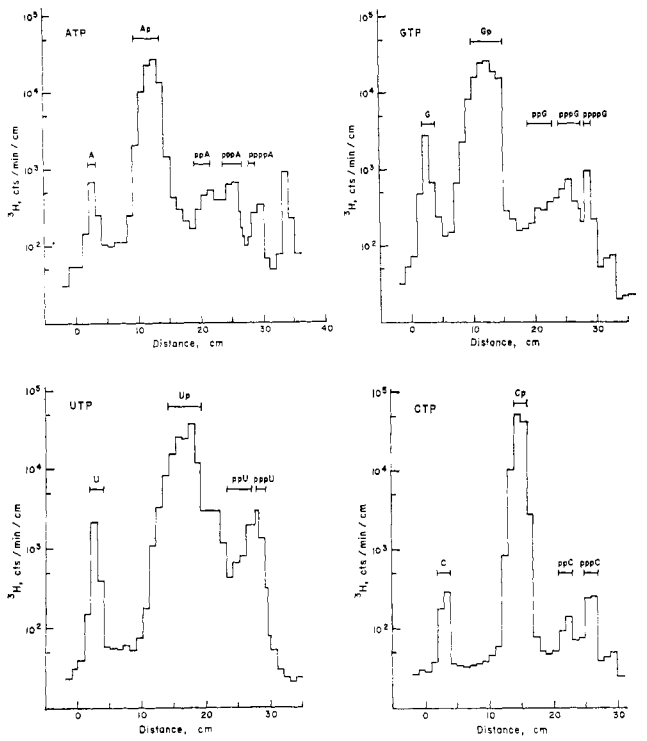

FIGURE 1: Electrophoretic distribution of alkaline hydrolysate of RNA transcribed from native calf thymus DNA by calf thymus RNA polymerase. Conditions for assay, alkaline hydrolysis, and electrophoresis are described in the Experimental Section. The appropriate marker compounds are indicated. Markers were electrophoresed simultaneously with the sample, except for ppppA, which in some experiments was run in parallel. In each case, the indicated nucleoside triphosphate was ^{3}H labeled in the reaction mixture. The number of cpm under each peak was totalled, after subtracting 30 cpm/strip as background. To obtain initiation values, cpm in the tetraphosphate peak were divided by the sum of cpm in the tetraphosphate, monophosphate, and nucleoside peaks (representing total incorporation into RNA). This gives the percentage of a given nucleotide which is the initial nucleotide. To determine the amount of initiation contributed by that nucleotide to the total RNA, the initiation percentage obtained above was multipled by the percentage of the respective nucleotide present in the RNA synthesized on a native DNA template (Keshgegian and Furth, 1972). For example, in the case of ATP, there are 666 cpm present as the tetraphosphate, and 80,000 cpm total incorporation into RNA. Initiation is thus 0.83% of total ATP incorporated. Since the RNA synthesized contains 21.5% ATP (Keshgegian and Furth, 1972), the contribution by ATP to total initiation is 0.18%. Total initiation (expressed as per cent of total RNA synthesized) is thus the sum of the four contributing percentages. Termination is the cpm in the nucleoside peak divided by the total cpm in the three peaks, similarly normalized according to the nucleotide composition of the RNA. The total number of cpm in RNA, initial nucleotide, and terminal nucleoside in each electropherogram are respectively: ATP, 80,000, 666, 986; UTP, 130,000, 106, 2694; GTP, 119,000, 1095, 4138; CTP, 108,000, 45, 416. Normalized values for initiation and termination are (%): ATP, 0.18, 0.26; UTP, 0.019, 0.48; GTP, 0.26, 0.98; CTP, 0.011, 0.10.

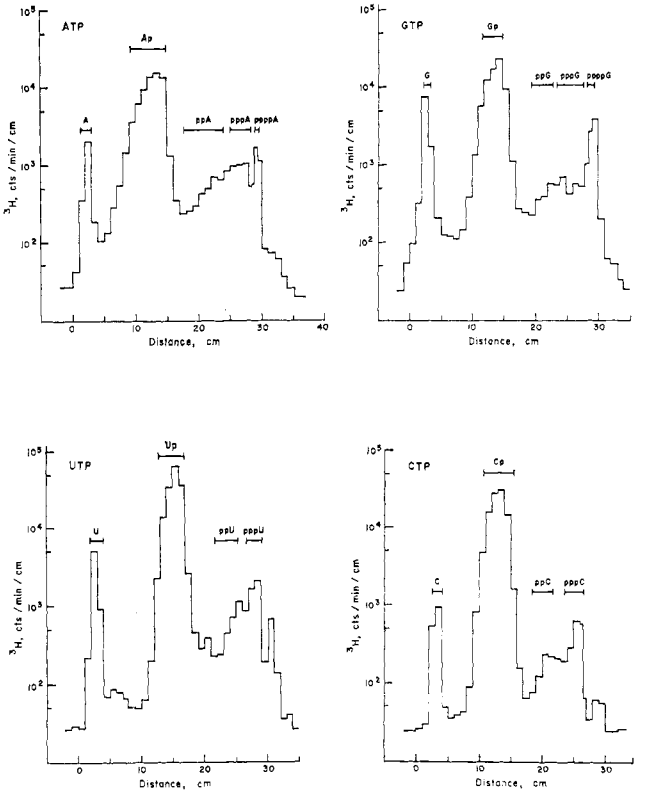

FIGURE 2: Electrophoretic distribution of alkaline hydrolysate of RNA transcribed from denatured calf thymus DNA by calf thymus RNA polymerase. See legend to Figure 1 for method of data analysis. Markers were electrophoresed simultaneously with the samples. The nucleotide composition of RNA transcribed from native DNA was also used as the normalization factor for RNA transcribed from a denatured DNA template, since there are not significant differences in nucleotide composition of RNA transcribed from the two templates (A. A. Keshgegian and J. J. Furth, unpublished observations). The total number of cpm in RNA, initial nucleotide, and terminal nucleoside in each electropherogram are respectively: ATP, 70,000, 1530, 2615; UTP, 157,000, 843, 6175; GTP, 86,900, 5933, 9700; CTP, 99,700, 70, 1467. Normalized values for initiation and termination are (%): ATP, 0.47, 0.81; UTP, 0.12, 0.92; GTP, 1.92, 3.1; CTP, 0.019, 0.40.

In the case of UTP, with a native DNA template, a separate tetraphosphate peak could not be resolved on three attempts (Figure 1). Therefore, counts/min in the leading edge of the first radioactivity peak, migrating ahead of the UTP marker, were considered to represent initiation by UTP. Separate uridine tetraphosphate peaks were resolved when denatured DNA or chromatin were used as template (Figures 2 and 3).

An even faster migrating peak of radioactivity was occasionally observed, especially with [^{3}H]ATP. Since it appeared inconstantly, did not comigrate with any standards, and represented few counts per minute, it was not further characterized.

There was also radioactivity migrating reproducibly in the nucleoside diphosphate and triphosphate regions of the electropherograms. This material was usually greater with mammalian than with bacterial enzyme products. Because the mammalian enzyme is less pure, protein in the preparation may be expected to trap more radioactive nucleotide in the acid precipitation step of the assay. Independent experiments have shown, in fact, that mammalian enzyme traps more counts per minute than bacterial enzyme. However, the possibility of some breakdown of tetraphosphate in the course of reaction or subsequently cannot be ruled out. Since the triphosphate is stable to alkaline hydrolysis and hydrolyzed a maximum of one-third by the reaction mixture (see Experimental Section), it is unlikely that the tetraphosphate would have been significantly degraded.

Initiation with Calf Thymus DNA as Template. Figure 1 shows that initiation with calf thymus RNA polymerase using a native DNA template occurs predominantly with ATP and GTP and that initiation by GTP is somewhat greater than by ATP. These results are confirmed in Table I, which shows an average of several determinations. Each assay and alkaline

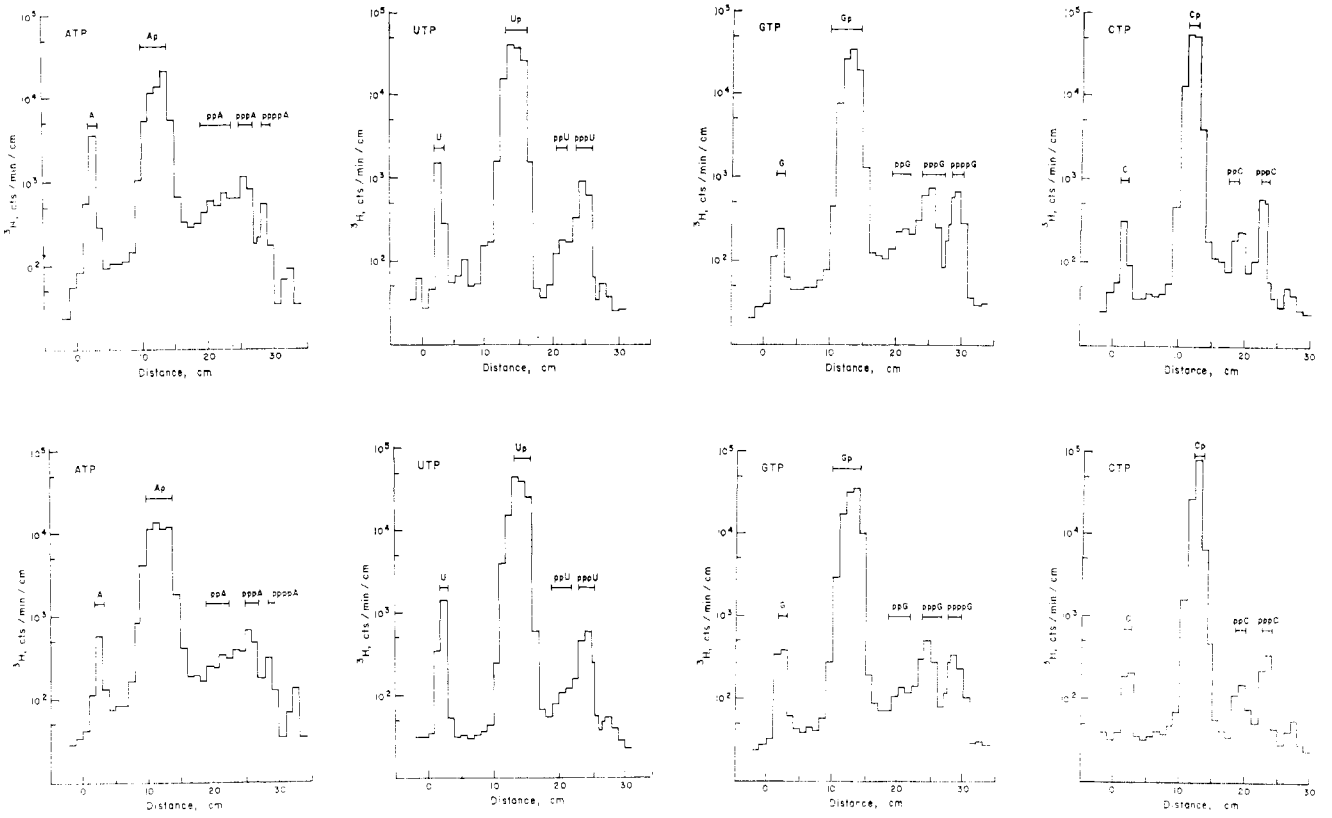

FIGURE 3: Electrophoretic distribution of alkaline hydrolysate of RNA transcribed from calf thymus chromatin by calf thymus and *E. coli* RNA polymerases: top row, calf thymus RNA polymerase; bottom row, *E. coli* RNA polymerase. See legend to Figure 1 for method of data analysis. Values were normalized according to the nucleotide composition of RNA transcribed from calf thymus chromatin by calf thymus and *E. coli* RNA polymerases (Keshgegian and Furth, 1972). The total number of cpm in RNA, initial nucleotide, and terminal nucleoside are respectively: (calf thymus enzyme) ATP, 65,800, 768, 4510; UTP, 122,000, 43, 1880; GTP, 92,100, 1400, 352; CTP, 126,000, 50, 392; (*E. coli* enzyme) ATP, 55,600, 467, 759; UTP, 127,000, 53, 1320; GTP, 99,300, 761, 728; CTP, 117,000, 57, 349. Normalized values for initiation and termination are (%): (calf thymus enzyme) ATP, 0.26, 1.52; UTP, 0.007, 0.33; GTP, 0.44, 0.10; CTP, 0.010, 0.079; (*E. coli* enzyme) ATP, 0.24, 0.40; UTP, 0.009, 0.23; GTP, 0.17, 0.16; CTP, 0.012, 0.076.

hydrolysis was performed in quadruplicate, and processed in duplicate. Therefore, each value in the table represents at least two electrophoretic determinations of the products of two different reaction mixtures. Initiation is over 90% by ATP and GTP relative to UTP and CTP. Table I also shows results of *E. coli* RNA polymerase with a native DNA template for comparison. These data are in basic agreement with previously reported values (Maitra and Hurwitz, 1965).

The results show that mammalian RNA polymerase, like the bacterial enzymes, initiates predominantly with purine nucleoside triphosphates. The results also suggest that, with native DNA template, there may be more initiation with ATP relative to GTP with calf thymus polymerase than with *E. coli* polymerase. However, the variation in the experimental values precludes a definitive statement.

Initiation by calf thymus RNA polymerase using a denatured calf thymus DNA template is illustrated in Figure 2, with averaged results in Table I. As has been observed for a variety of templates with RNA polymerase from *E. coli* (Maitra and Hurwitz, 1965), *A. vinelandii* (Krakow and Horsely, 1967), and *M. luteus* (Frederick *et al.*, 1969) there is relatively greater total initiation (although still over 90% with ATP and GTP) and initiation by GTP relative to ATP increases. Increased total initiation indicates a shorter average chain length. If we take the reciprocal of the total initiation (0.55%) with native DNA, we find that the average chain length is 182 nucleotides. Using denatured DNA, total initiation is 2.47%, which gives a chain length of 40. Thus the RNA is approximately one-fourth as long when denatured DNA is used as template. (These numbers do not necessarily reflect completed chains, since RNA synthesis is not complete when the reaction is terminated, and since reinitiation may have occurred.)

With *E. coli* RNA polymerase initiation also increases upon denaturation of the DNA template, as has been reported (Maitra and Hurwitz, 1965), although in our system, initiation by GTP relative to ATP does not increase and in fact may decrease. This latter observation is in disagreement with the results of Maitra and Hurwitz (1965), which were obtained under low salt conditions. However, Maitra *et al.* (1967) showed that salt concentration can alter the relative amounts of initiation by ATP and GTP. Maitra and Barash (1969) found that initiation by GTP in the presence of 0.2 M KCl increases upon denaturation of a T2 DNA template. Initiation by *E. coli* enzyme with denatured calf thymus DNA has not been studied under high salt (0.2 M KCl) conditions.

Our results indicate that calf thymus RNA polymerase initiates with purine nucleoside triphosphates, and that denaturation of the template results in greater initiation by GTP relative to ATP. Preliminary data reported by Chambon *et al.* (1970) give similar initiation values for form B (α-amanitin sensitive) calf thymus RNA polymerase, determined by incorporation of γ-^{32}P-labeled nucleoside triphosphates. These investigators found that form A (α-amanitin resistant) RNA polymerase initiates only with GTP using a native calf thymus DNA template. Initiation with ATP does not occur or is too

TABLE I: Initiation of RNA Synthesized *in Vitro*.[a]

Template	pppAp (%)	pppGp (%)	pppUp (%)	pppCp (%)	A/G
	Calf Thymus RNA Polymerase				
Native DNA	0.21 ± 0.03 (3)	0.29 ± 0.03 (2)	0.038 ± 0.020 (3)	0.012 ± 0.001 (2)	0.72 ± 0.13
Denatured DNA	0.54 ± 0.06 (2)	1.80 ± 0.10 (2)	0.11 ± 0.01 (2)	0.020 ± 0.002 (2)	0.30 ± 0.04
Chromatin	0.23 ± 0.06 (4)	0.44 ± 0.01 (2)	0.010 ± 0.003 (2)	0.009 ± 0.001 (2)	0.52 ± 0.14
	E. coli RNA Polymerase				
Native DNA	0.17 ± 0.05 (4)	0.39 ± 0.17 (4)	0.021 ± 0.004 (2)	0.008 ± 0.001 (2)	0.44 ± 0.23
Denatured DNA	0.79 ± 0.17 (3)	0.90 ± 0.01 (2)	0.047 ± 0.003 (2)	0.024 ± 0.005 (2)	0.88 ± 0.20
Chromatin	0.28 ± 0.04 (4)	0.18 ± 0.02 (2)	0.010 ± 0.001 (2)	0.008 ± 0.004 (2)	1.56 ± 0.28

[a] Values are expressed as per cent initiation relative to total incorporation into RNA of each nucleotide and are corrected for the nucleotide composition of the RNA (see legends to Figures 1–3). The number in parentheses after each value indicates the number of electrophoretic determinations which were averaged to give that value. A/G indicates the ratio of initiation by pppAp relative to pppGp for each template.

TABLE II: Termination of RNA Synthesized *in Vitro*.[a]

Template	A (%)	G (%)	U (%)	C (%)
	Calf Thymus RNA Polymerase			
Native DNA	0.39 ± 0.16 (20)	1.01 ± 0.03 (52)	0.46 ± 0.04 (23)	0.10 ± 0.01 (5)
Denatured DNA	1.10 ± 0.30 (23)	2.20 ± 0.90 (47)	0.95 ± 0.03 (20)	0.47 ± 0.05 (10)
Chromatin	1.19 ± 0.24 (66)	0.20 ± 0.10 (11)	0.33 ± 0.01 (19)	0.08 ± 0.01 (4)
	E. coli RNA Polymerase			
Native DNA	0.38 ± 0.22 (17)	1.54 ± 0.12 (68)	0.27 ± 0.04 (12)	0.068 ± 0.001 (3)
Denatured DNA	0.61 ± 0.02 (13)	3.24 ± 0.07 (69)	0.66 ± 0.01 (14)	0.21 ± 0.02 (4)
Chromatin	0.53 ± 0.09 (50)	0.22 ± 0.04 (21)	0.24 ± 0.01 (23)	0.070 ± 0.005 (7)

[a] Values are expressed as per cent termination relative to total incorporation into RNA of each nucleotide, and are corrected for the nucleotide composition of the RNA (see legends to Figures 1–3). Each value represents an average of at least two electrophoretic determinations. The number in parentheses after each value indicates the per cent of termination by each nucleoside relative to total termination for the given template and enzyme.

infrequent to be detectable. Our results agree more with those of the B polymerase of Chambon *et al.*, even though our enzyme is resistant to low levels of α-amanitin. Nevertheless, our enzyme shares other characteristics with form B (or form II of Roeder and Rutter, 1969), such as elution from DEAE-Sephadex at 0.23 M $(NH_4)_2SO_4$. It may thus represent an intermediate form. Since the RNA polymerase preparation is obtained by low-salt extraction of a whole-cell homogenate (Furth *et al.*, 1970), it is also possible that the enzyme is a cytoplasmic form or readily leached out of the nucleus. Seifart *et al.* (1972) have reported the isolation of a possible cytoplasmic RNA polymerase, with intermediate sensitivity to α-amanitin, from rat liver.

Initiation with Calf Thymus Chromatin as Template. Initiation with calf thymus and *E. coli* RNA polymerases using a chromatin template is shown in Figure 3 and Table I. Over 95% of initiation using both enzymes occurs with ATP and GTP. There is a marked difference of initiation by ATP relative to GTP in comparing the two enzymes. With calf thymus polymerase initiation may occur slightly less with ATP relative to GTP using chromatin, rather than native DNA, as template. With *E. coli* polymerase, however, initiation by ATP relative to GTP is markedly higher using chromatin, rather than native DNA, as template. For calf thymus enzyme, total amount of initiation is slightly higher than with a native DNA template (*i.e.*, the RNA is slightly shorter). Total initiation is slightly lower than with a native DNA template for *E. coli* enzyme, *i.e.*, the RNA is slightly longer. However, these results are not statistically significant.

Thus there appears to be a striking difference in the relative amount of initiation by ATP and GTP between the two enzymes with a chromatin template, but not a native DNA template, while initiation by purine nucleoside triphosphates relative to pyrimidine nucleoside triphosphates and RNA length do not significantly differ.

The enzyme preparations used in this study are partially purified. It is conceivable that different values for initiation by ATP relative to GTP may be obtained with more highly purified enzyme fractions. We have not been able to investigate this question, since it has not been possible to obtain sufficient quantities of more refined preparations. However, it should be noted that changes in the amount of initiation by ATP relative to GTP are observed for the same enzyme when chromatin rather than DNA is used as template. This suggests that some factor in the chromatin rather than an enzyme impurity is affecting initiation. Similarly, the effect of chromatin is different for the two enzymes. For *E. coli* RNA polymerase initiation is higher with ATP using a chromatin tem-

plate; for calf thymus polymerase initiation is slightly higher with GTP. This indicates that the effect of chromatin on initiation is specific, and not due to a contaminating enzyme activity in the chromatin.

Therefore the difference in initiation by ATP relative to GTP between the two enzymes on a chromatin template suggests that the enzymes are initiating at different sites on chromatin. This implies that factors in chromatin interact differently with the mammalian and bacterial enzymes, and direct them to different sites for transcription. The selection of a given set of genes for transcription in the differentiated cell may thus involve not only the interaction of chromatin repressor or derepressor factors with the DNA, but also their specific interaction with the appropriate RNA polymerase. Butterworth *et al.* (1971), using a different approach, have also concluded that mammalian and bacterial RNA polymerases may transcribe at different sites on chromatin.

Termination of RNA Synthesis. Electrophoresis also resolves the terminal nucleoside in the alkaline hydrolysate of RNA, as illustrated in Figures 1–3. In our system, when the reaction is terminated there are both complete RNA chains and those still being synthesized; termination with all four nucleosides would be expected, but with more radioactivity in the nucleosides at the end of the complete RNA chain. This type of distribution is in fact observed for both enzymes. Average data are presented in Table II. With native or denatured DNA as template, termination occurs in all nucleosides, with a high percentage in guanosine. This is in contrast to the preferential termination in uridine found with *E. coli* RNA polymerase and bacteriophage DNA as template (Maitra *et al.*, 1970; Millette *et al.*, 1970). However, Mueller and Bremer (1969) did not find preferential termination with uridine in their system, using *E. coli* RNA polymerase and T4 DNA.

When chromatin rather than DNA is used as template, there is a marked shift to termination with adenosine. For calf thymus polymerase, while termination with adenosine is $20 \pm 8\%$ using native DNA as template, it is $66 \pm 16\%$ using chromatin. For *E. coli* polymerase the values are $17 \pm 10\%$ with native DNA, and $50 \pm 10\%$ with chromatin.

Termination results must be interpreted cautiously, since total termination is two to four times higher than total initiation. This phenomenon has been observed previously. Mueller and Bremer (1969), using *E. coli* enzyme, stated that the enzyme may occasionally skip formation of an internucleotide bond after pyrophosphate release. As a result some RNA chains would be produced with a 5′-end nucleoside monophosphate, which would be converted to the nucleoside diphosphate (pNp) upon alkaline hydrolysis. Thus the total initiation represented by tetraphosphate and diphosphate after alkaline hydrolysis would equal total termination. Maitra *et al.* (1967) reported a small but significant degradation of monophosphate to nucleoside during alkaline hydrolysis.

If possible degradation is approximately the same for all four nucleosides, it may be concluded that preferential termination occurs with adenosine using chromatin, but not DNA, as template. Our preparation of calf thymus RNA polymerase does not synthesize poly(A) in the presence of all four nucleoside triphosphates (Keshgegian and Furth, 1972). Furthermore, RNA synthesized by the mammalian enzyme with chromatin as template is similar in nucleotide composition to RNA synthesized with DNA as template (Keshgegian and Furth, 1972). Thus, it is unlikely that poly(A) is being added to the RNA synthesized from chromatin, although the presence of a small amount of poly(A) polymerase activity in our chromatin preparation cannot be excluded (Edmonds and Abrams, 1960). Tsiapalis *et al.* (1973) have purified a form of poly(A) polymerase from calf thymus which is stimulated by oligo(A) primers. RNA transcribed from chromatin may, therefore, preferentially terminate in a single A [or oligo(A)] in order to provide an efficient primer for poly(A) polymerase. If this be true, then specific termination signals may be acting in chromatin *in vitro*. This possibility is presently under investigation.

Acknowledgments

We thank Nora Zuno, Sherry Meltzer, and Irene Andrulis for excellent technical assistance.

References

Bekhor, I., Kung, G. M., and Bonner, J. (1969), *J. Mol. Biol. 39*, 351.

Bremer, H., Konrad, M. W., Gaines, K., and Stent, G. S. (1965), *J. Mol. Biol. 13*, 540.

Butterworth, P. H. W., Cox, R. F., and Chesterton, C. J. (1971), *Eur. J. Biochem. 23*, 229.

Chambon, P., Gissinger, F., Mandel, J. L., Jr., Kedinger, C., Gniazdowski, M., and Meihlac, M. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 693.

Edmonds, M., and Abrams, R. (1960), *J. Biol. Chem. 235*, 1142.

Frederick, E. W., Maitra, U., and Hurwitz, J. (1969), *J. Biol. Chem. 244*, 413.

Furth, J. J., and Austin, G. E. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 641.

Furth, J. J., Nicholson, A., and Austin, G. E. (1970), *Biochim. Biophys. Acta 213*, 124.

Furth, J. J., and Pizer, L. I. (1966), *J. Mol. Biol. 15*, 124.

Huang, R. C. C., and Huang, P. C. (1969), *J. Mol. Biol. 39*, 365.

Keshgegian, A. A., and Furth, J. J. (1972), *Biochem. Biophys. Res. Commun. 48*, 757.

Keshgegian, A. A., and Garibian, G. S. (1973), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 32*, 1795.

Krakow, J. S., and Horsley, W. J. (1967), *J. Biol. Chem. 242*, 4796.

Maitra, U., and Barash, F. (1969), *Proc. Nat. Acad. Sci. U. S. 64*, 779.

Maitra, U., and Hurwitz, J. (1965), *Proc. Nat. Acad. Sci. U. S. 54*, 815.

Maitra, U., Lockwood, A. H., Dubnoff, J. S., and Guha, A. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 143.

Maitra, U., Nakata, Y., and Hurwitz, J. (1967), *J. Biol. Chem. 242*, 4908.

Millette, R. L., Trotter, C. D., Herrlich, P., and Schweiger, M. (1970), *Cold Spring Harbor Symp. Quant. Biol. 35*, 135.

Mueller, K., and Bremer, H. (1969), *J. Mol. Biol. 43*, 89.

Paul, J., and Gilmour, R. S. (1968), *J. Mol. Biol. 34*, 305.

Roeder, R. G., and Rutter, W. J. (1969), *Nature (London) 224*, 234.

Seifart, K. H., Benecke, B. J., and Juhasz, P. P. (1972), *Arch. Biochem. Biophys. 151*, 519.

Tsiapalis, C. M., Dorson, J. W., De Sante, D. M., and Bollum, F. J. (1973), *Biochem. Biophys. Res. Commun. 50*, 737.