Proc. Nat. Acad. Sci. USA
Vol. 72, No. 3, pp. 1082–1086, March 1975

Chromatin Directed Transcription of 5S and tRNA Genes

(RNA synthesis/myeloma)

WILLIAM F. MARZLUFF, JR.* AND RU CHIH C. HUANG

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Communicated by Donald D. Brown, December 26, 1974

ABSTRACT **Chromatin prepared by gentle methods from mouse myeloma cells retained its ability to synthesize RNA using bound endogenous RNA polymerase (RNA nucleotidyltransferase; nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6). The transcription resembles that observed *in vivo* in several respects. The low-molecular-weight RNA species, 5S RNA and the 4.5S precursor to 4S RNA, are transcribed accurately and transcription is reinitiated continually *in vitro*. Their synthesis was not inhibited by α-amanitin (1 μg/ml) as was found previously for these species in isolated nuclei.**

Several chromatin-transcription systems for specific genes were described recently (1–6). Using purified globin cDNA as the probe and *Escherichia coli* RNA polymerase (RNA nucleotidyltransferase; nucleosidetriphosphate:RNA nucleotidyltransferase, EC 2.7.7.6), Axel, *et al.* (1) and Gilmour and Paul (2) found that globin sequences were transcribed from the chromatin of erythroid tissues but not from the chromatin of other systems. Steggles *et al.* (3) have extended these studies to show that homologous RNA polymerase also transcribes the globin sequences and these constitute a slightly larger percentage of the total product than when *E. coli* RNA polymerase is used. Astrin (4) and Shih *et al.* (5) showed that *E. coli* RNA polymerase transcribed SV40 viral sequences using chromatin from SV40 virus-transformed cells as template. The same strand of SV40 DNA was transcribed both *in vivo* and *in vitro*, whether DNA or chromatin was used as template (5). Most recently, Jacquet *et al.* (6) demonstrated the *in vitro* synthesis of avian myeloblastosis viral RNA sequences by nuclei and chromatin isolated from viral-infected chick myeloblasts.

Such studies, while indicating the accessibility of specific genes for transcription in chromatin, give little information about the precision of chain initiation, termination, and correct DNA strand selection. Indeed, several lines of evidence suggest that there are differences between *in vivo* and *in vitro* transcription. Using chromatin from *Xenopus laevis* cells, Reeder (7) has shown that exogenous RNA polymerase does not transcribe faithfully the genes for rRNA and 5S RNA. Both strands of DNA as well as the "spacer" regions, which normally are not transcribed *in vivo* are transcribed *in vitro*. In addition, while *in vivo* these genes are transcribed by specific RNA polymerases, both Xenopus RNA polymerase I and II, as well as *E. coli* RNA polymerase, transcribed the chromatin identically *in vitro* (8). It seems then, that factors which exercise fine control over *in vivo* transcription have been lost during the isolation of either chromatin or of RNA polymerase.

* Present address: Department of Chemistry, Florida State University, Tallahassee, Fla. 32306.

In facing such concerns, we have concentrated our efforts on developing an *in vitro* synthesis system which hopefully will yield the same transcriptional products as are found in living cells. We have reported previously that myeloma nuclei are capable of synthesizing RNA at a high rate (9, 10). The RNA product resembles nuclear RNA with respect to size range as determined by sucrose gradient centrifugation and contains the same specific RNA species as analyzed by gel electrophoresis. In particular, these nuclei reinitiate continually the synthesis of 5S RNA and of 4.5S precursor to tRNA. Other workers have reported faithful transcription of rRNA in isolated nuclei (11, 12) and of viral-specific RNAs in nuclei from virus-infected cells (13, 14).

As a step further, we report here the isolation of myeloma chromatin with high endogenous RNA polymerase activity. The chromatin faithfully synthesizes specific RNA species, and 5S genes are transcribed continually *in vitro* with correct DNA strand selection. This chromatin, which contains only half of the nuclear protein and 10% of the nuclear RNA, promises to be useful for the study of the control of specific gene expression in eukaryotes.

MATERIALS AND METHODS

Cells. The cells used were 66-2 mouse myeloma cells which produce a K-chain. They were grown to a concentration of 3 to 5 × 10^5/ml (9, 10).

Preparation of Chromatin. Nuclei were prepared exactly as described previously (9). The nuclei were suspended in incubation medium (25% glycerol, 5 mM Mg^{++}, 5 mM dithiothreitol, 50 mM Tris·HCl at pH 8) at a concentration of 4 to 8 × 10^7 nuclei per ml (0.4–0.8 mg of DNA per ml) and adjusted to 0.12 M KCl with 2 M KCl. After 3 min at 4° they were centrifuged at 1000 rpm for 3 min. The nuclear pellet was suspended in 10% glycerol, 0.01 M Tris·HCl at pH 8, 1 mM dithiothreitol, and recentrifuged.

The nuclei were broken during the centrifugation and the chromatin pellet was suspended with a glass rod in 10% glycerol, 0.01 M Tris·HCl at pH 8, 1 mM dithiothreitol, and recentrifuged. This washing procedure was repeated three to five times or until the chromatin swelled. The chromatin gel, at a concentration of 300–700 μg of DNA per ml, was sheared by passing through a 16-gauge needle from a 1 ml syringe 10 times, and then through a 23-gauge needle five times. Less than 5% contamination by unbroken nuclei was found microscopically. The UV absorption spectra, protein:DNA ratio (1.9:1–2.1:1) and electrophoretic protein pattern of the chromatin was very similar to that found for myeloma chromatin prepared by Murphy *et al.* (15). The RNA components of

chromatin prepared in this way were identical with these found in myeloma chromatin prepared by other methods (manuscript in preparation). Essentially all the ribosomal and preribosomal RNA was present in the initial wash of the nuclei in 0.12 M KCl. Forty to 50% of the nuclear protein and 90% of the RNA was removed during the washing procedures. Inclusion either of 1 mM EDTA or 1 mM Mg^{++} in the washing buffer did not affect the activity. The chromatin was used immediately since it loses activity within 3 hr at 4° and on freezing.

Assay of RNA Synthesis. Conditions for RNA synthesis were as previously described (10). Chromatin was incubated at 25° in a solution containing 10% glycerol, 25 mM Tris·HCl at pH 8, 5 mM Mg^{++}, 1 mM Mn^{++}, 0.12 M KCl, 0.4 mM each of ATP, UTP, CTP, 0.04 mM [^{3}H]GTP (10 Ci/mmol) and chromatin (200–500 μg of DNA per ml). The reaction was terminated with 5 volumes of 1% sodium dodecyl sulfate in 10 mM EDTA. Trichloroacetic acid precipitable counts were determined as described previously (9).

RNA was prepared from the reaction mixture exactly as described previously using sodium dodecyl sulfate-phenol at pH 5. The conditions of analysis of RNA by sucrose gradient centrifugation and gel electrophoresis have been described (9).

RNA·DNA hybridization to purified *Xenopus laevis* 5S DNA prepared as described by Brown *et al.* (16) was carried out as described previously (10).

For study of initiation of RNA synthesis, [γ-^{32}P]GTP was synthesized by the method of Glynn and Chappell (17). An important final step in the purification of [γ-^{32}P]GTP was to pass the preparation over a Norit column in 0.2 M HCl, to wash the Norit with water and elute the GTP with 3% NH_4OH–70% ethanol. This step separated the product GTP from the contaminants in the commercial ^{32}P preparation. The GTP was dried, dissolved in water, lyophilized, and then dissolved in water before use. Chromatin was incubated with both [γ-^{32}P]-GTP and [^{3}H]GTP. The RNA was purified as usual with the inclusion of a gel filtration step (Sephadex G-25, 1 M NaCl) to further remove unincorporated precursors prior to sucrose gradient centrifugation and gel electrophoresis. To identify pppGp, the RNA was digested with ribonuclease T_1 (Calbiochem) and the digest chromatographed on polyethylenimine impregnated-cellulose thin-layer plates (Brinkmann) and developed with 1.5 M KH_2PO_4 at pH 3.5 as described by Cashel and Kalbacher (18).

RESULTS

Effect of conditions of chromatin isolation on *in vitro* RNA synthesis

Isolated chromatin incorporated substantial amounts of [^{3}H]GTP into RNA when incubated at 25° (Fig. 1). The activity of the chromatin was dependent on the conditions of isolation. The greatest activity was obtained when chromatin was isolated by extraction of nuclei with 0.1–0.15 M KCl, and less activity was obtained when no KCl was included in the extraction. When the KCl concentration was raised to 0.35 M, the activity of the chromatin was essentially abolished although all the RNA polymerase remained with the chromatin. Under the normal extraction conditions (0.12 M KCl) 40–50% of the nuclear protein and 90% of the nuclear RNA was removed. No increase in activity was found when the extract was added back to the chromatin isolated by the normal extraction procedure. No RNA polymerase activity was detectable in the extract when DNA was mixed with the extract. The RNA synthetic activity of the chromatin varied from 20–80% of that of the intact nuclei. In all cases, however, the chromatin synthesized RNA for at least 30 min. The activity

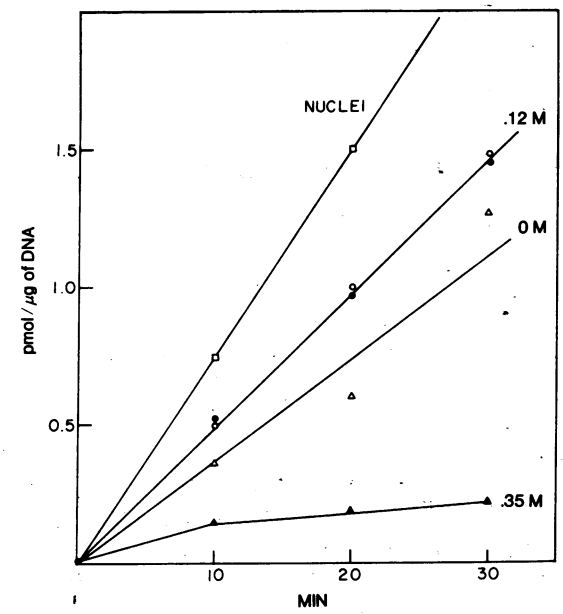

FIG. 1. Synthesis of RNA by chromatin. Chromatin was prepared by different methods and RNA synthesis measured as described in *Materials and Methods*. Identical conditions at 25° were used for all assays. The salt concentration in the extraction of nuclei during chromatin preparation was varied. One pmol of GMP incorporated 4000 cpm. Four micrograms of DNA were present in each 0.02 ml assay. □——□, nuclei, ○——○, chromatin prepared by extraction with 0.12 M KCl, ●——●, chromatin prepared by extraction with 0.12 M KCl and assayed in the presence of material extracted by 0.12 M KCl, △——△, chromatin prepared by extraction with 0.01 M Tris·HCl, ▲——▲, chromatin prepared by extraction with 0.35 M KCl.

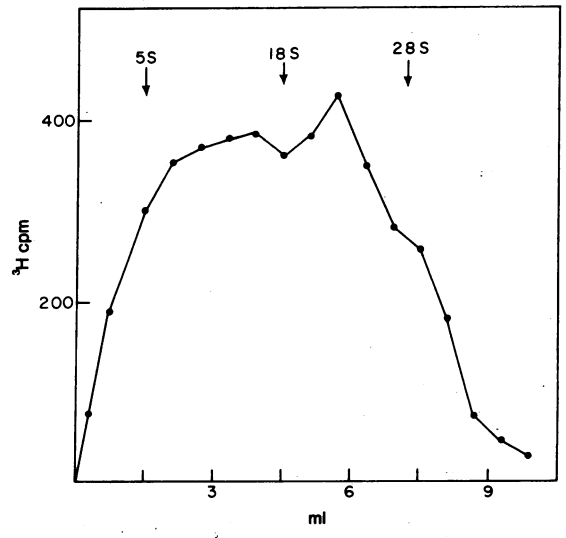

FIG. 2. Analysis of RNA product by sucrose gradient centrifugation. RNA was purified from a chromatin-primed synthesis and analyzed by centrifugation in 10–70% sucrose gradients (9). Centrifugation was at 22,000 rpm in the SW25.3 rotor for 18 hr at 25°; 0.6 ml fractions were collected and 0.03 ml aliquots analyzed for trichloroacetic acid precipitable counts.

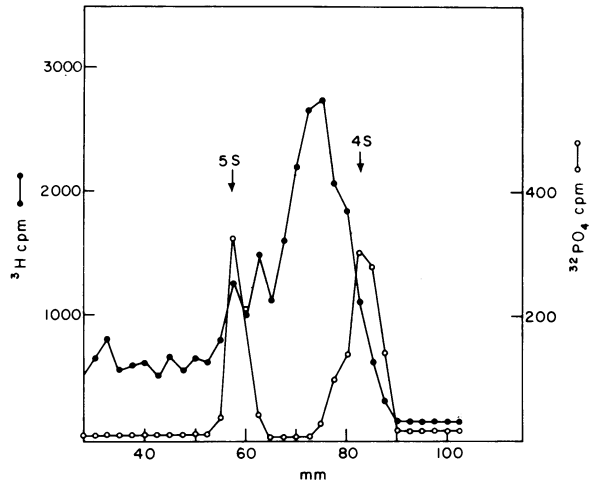

FIG. 3. Analysis of RNA by gel electrophoresis. 4–8S RNA (fractions 2–5, Fig. 2) were analyzed by electrophoresis in 10% polyacrylamide gels (10). As a marker ^{32}P-labeled 5S rRNA and 4S tRNA were added to the samples. Gels were sliced into 1.8 mm sections and counted. ●——●, ^{3}H *in vitro* RNA; ○——○, ^{32}P marker RNA.

of the chromatin was very unstable when compared to the activity of isolated nuclei and lost activity on freezing and thawing. The chromatin, like the nuclei, was much more active at 25° than at 37°.

Analysis of chromatin-primed RNA product

The RNA was prepared after synthesis by extraction with phenol–sodium dodecyl sulfate and analyzed by sucrose density gradient centrifugation. The RNA was extremely heterogeneous in size, ranging up to 28 S (Fig. 2). This is significantly smaller than the RNA made in isolated nuclei which ranged up to 45 S in size (9). The smaller size of the RNA products may have been due to the slight shearing of template chromatin during isolation. The actual size of the DNA, however, was not measured.

When the low-molecular-weight RNA was analyzed by gel electrophoresis (Fig. 3), specific RNA species were observed which were identical to 5S RNA and the 4.5S precursor to 4S RNA. Unlike isolated nuclei which synthesized some 4S RNA *in vitro* due to conversion of 4.5S RNA to tRNA (10), the chromatin in eight different experiments synthesized no 4S RNA, thus indicating that the enzyme responsible for the conversion was extracted during preparation of the chromatin.

The extended synthesis of 5S and 4.5S RNA suggested that these species were being reinitiated faithfully and transcribed continually *in vitro*. To test this, chromatin was incubated simultaneously with [γ-^{32}P]GTP and [^{3}H]GTP. On analysis

TABLE 1. *5S RNA synthesized*

Condition	Time (min)	Total synthesis (%)
*In vivo**	30	0.8–1.0
By nuclei*	30	0.25–0.6
By chromatin 0.12 M KCl	30	0.6–1.0
By chromatin 0.4 M KCl	30	<0.05
By chromatin 0.12 M KCl	5	
0.4 M KCl	25	0.1–0.2

* Data obtained from ref. 10.

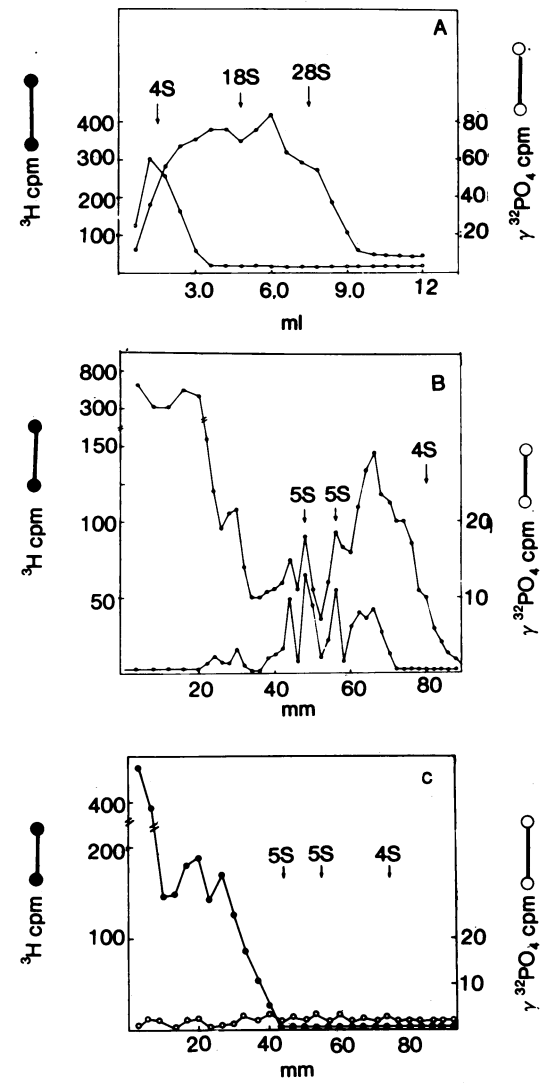

FIG. 4. Initiation of low-molecular-weight RNA *in vitro*. Chromatin was incubated in the presence of both [^{3}H]GTP (3 Ci/mmol) and [γ-^{32}P]GTP (2.5 Ci/mmol) at low salt (0.15 M KCl, A and B) and high salt (0.4 M KCl, C). RNA was analyzed by sucrose gradient centrifugation (A) and 0.08 ml of each 0.6 ml fraction analyzed for trichloroacetic acid precipitable counts. 4–8S RNA fraction was further examined by electrophoresis in 10% polyacrylamide gel (B) as in Fig. 3. An aliquot of the same 4–8S sample was also analyzed for the presence of pppGp (Fig. 5). RNA synthesis was carried out in the presence of 0.4 M KCl (C). The position of both conformers of 5 S is indicated.

by sucrose gradients, ^{32}P was only incorporated into 4–8S RNA, (Fig. 4A). This RNA was further analyzed by acrylamide gel electrophoresis (Fig. 4B). Much of the ^{32}P in the gel was associated with both conformers of 5S RNA (10) and with 4.5S RNA. The two forms of 5S RNA have also been observed by Weinberg and Penman and others (24).

When the reaction was carried out in 0.4 M KCl, there was significant RNA synthesis although no 5S or 4.5S RNA was detected by acrylamide gel electrophoresis (Fig. 4C). Under the high salt condition, RNA polymerase is unable to bind and to initiate new RNA chains although continuation of chain elongation was not affected. An extensive study on this point with bacterial RNA polymerase on DNA was reported by Hyman and Davidson (19), and on chromatin by Cedar and Felsenfeld (20).

TABLE 2. *Hybridization of chromatin-primed RNA to 5S DNA**

DNA strand	Input		Bound		5S competed		Efficiency	Amount of 5S RNA in 4–8S fraction†	
	^3H	^{32}P 5S	^3H	^{32}P 5S	^3H	^{32}P		Hybrid	Gel
5 S$^+$	6500	3000	35	230	6	20	7.7%	7%	8%
5 S$^-$	6500	3000	3	2	0	0			

* 4–8S RNA (20% of total RNA) from sucrose gradient fractionation of RNA (Fig. 2) was hybridized with separated strands of 5S DNA from *Xenopus laevis* as previously described. As an internal standard, ^{32}P-labeled 5S RNA was included in the reaction. RNA was synthesized by chromatin in the presence of α-amanitin (1 μg/ml). An aliquot was also analyzed by gel electrophoresis.

† Amount of 5S RNA in the sample was calculated both from the hybridization efficiency and by gel electrophoresis of an aliquot of the same sample. The results were representative of two independent experiments. Background was subtracted, i.e., for ^3H, the instrument background was 50 cpm and the blank filter background was 5–10 cpm; for ^{32}P, the instrument background was 20 cpm and the blank filter background was 0–5 cpm. The specific activity of the 5S RNA was 250,000 cpm/μg. One microgram of 5S DNA was on each filter (50 ng of 5S gene sequence).

To further indicate that synthesis of 5S and 4.5S RNA were being reinitiated continually, the reaction was carried out at 0.12 M KCl for 5 min and then adjusted to 0.4 M KCl for 25 min. The proportion of 5S product was much lower in this case than when the reaction was allowed to proceed for 30 min in 0.12 M KCl. This result further supports the conclusion that these RNAs were being continually synthesized during the whole incubation period under optimum conditions.

While the total amount of RNA synthesized from chromatin per μg of DNA was somewhat lower than that found with isolated nuclei, (Fig. 1), the proportion of the RNA that was 5 S was two to three times higher (0.6–1.0%) (Table 1). This may be due to shearing of the chromatin which might affect very large genes much more drastically than the smaller 5S genes. The synthesis of 5S RNA and 4.5S RNA was unaffected by a low concentration of α-amanitin (1 μg/ml) although total incorporation was reduced 30–40%, as was the case for the isolated nuclei (10, 21). This indicates that the 5S and tRNA genes are not transcribed by RNA polymerase II. We have not as yet examined the effect of using higher concentrations of α-amanitin on the synthesis of these RNA species.

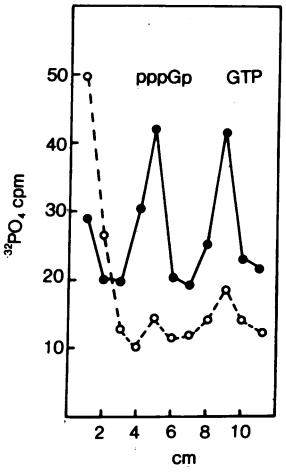

FIG. 5. Identification of pppGp. The RNA sample (4–8S fraction from Fig. 4A) was dissolved in 0.05 ml of water and 0.025 ml was analyzed by gel electrophoresis (Fig. 4B). The remaining RNA was divided into two aliquots; 0.015 ml was treated at 37° with 2 μg of T_1 ribonuclease and then chromatographed on polyethylenimine impregnated-cellulose developed with 1.5 M KH_2PO_4 at pH 3.4. The remaining 0.01 ml was chromatographed without treatment with T_1. The position of pppGp (prepared by reaction of the substrate with *E. coli* polymerase and DNA) and GTP is marked. ●——●, T_1 treated; O- - -O, not treated.

Identification of the 5S RNA product was also identified by specific hybridization with 5S DNA prepared from *Xenopus laevis* (Table 2). The RNA synthesized *in vitro* hybridized with only the plus strand of the 5S DNA. Hybridization was competed by purified 5S RNA. By including a ^{32}P standard of 5S RNA in the reaction to monitor hybridization efficiency the results can be quantitated. Seven to eight percent of the RNA synthesized by chromatin in the size range between 4–8 S was 5S RNA. This approximates to about 0.6–1% of the total RNA synthesized (Table 1).

Initiation of RNA synthesized *in vitro*

To examine where the ^{32}P from [γ-^{32}P]GTP was incorporated into 5S and 4.5S RNAs, we digested 4–8S RNA (Fig. 4A) with RNase T_1 and then analyzed the product by thin-layer chromatography. Much of the ^{32}P in the gel was identified as pppGp on the chromatogram, and essentially no counts were found as pppGp without T_1 digestion (Fig. 5). From the known specific activities of [^{32}P]GTP and [^3H]GTP in the reaction, and the number of guanosine residues in 5S RNA (1 terminal and 33 total) (22) the number of molecules terminated with labeled pppGp could be calculated. Eighty to 100% of the molecules were initiated *in vitro*†. This result is in contrast to that obtained with isolated nuclei, where only 10–20% of the 5 S contained labeled pppGp with the predominant 5′ termini being pGp (10). Presumably a phosphatase activity which converts the pppGp terminus to pGp might also be extracted during chromatin preparation.

DISCUSSION

The experiments reported here demonstrate that faithful RNA transcription continues *in vitro* with chromatin prepared by a gentle procedure. Despite removal of much of the nuclear protein and RNA, the chromatin retained RNA syn-

† The relative specific activity ($^{32}P/^3H$) was 5/6. The relative counting efficiencies (^{32}P = 100%, ^3H = 25%) was 4:1. Hence for an equal number of molecules, one expected a counting ratio of ^{32}P:^3H of 3.6:1. However, since only one ^{32}P-disintegration per 33 ^3H-disintegration would be expected with 5S RNA, a ^{32}P:^3H ratio of 0.12:1 for 100% initiation would be expected. The observed value was 0.14:1; it was calculated from the 5S peak of the gel electrophoresis data in Fig. 4B.

thetic properties similar to that of isolated nuclei. The major differences between the two systems were:

(1) The chromatin system was much less stable. Activity was lost rapidly at 4° and was completely inactivated by freezing. The activity of the chromatin ranged from 20 to 80% of that of isolated nuclei.

(2) The RNA product from chromatin was of lower average molecular weight than the product of isolated nuclei. The reason for this is not clear, but may be due to shearing of the DNA template during isolation. Much of the RNA produced by nuclei is rRNA which is normally synthesized in the nucleolus. The nucleolus may become disorganized during the preparation of chromatin, thus resulting in lower-molecular-weight RNA being synthesized. However the low-molecular-weight RNAs are synthesized accurately.

(3) No 4S RNA was produced by the chromatin while some is produced in isolated nuclei. This is undoubtedly due to extraction of the enzyme responsible for cleaving 4.5S RNA to 4S RNA during chromatin preparation.

However, the similarities between the chromatin and isolated nuclei were striking. 5S RNA was faithfully transcribed by a RNA polymerase activity insensitive to a low concentration of α-amanitin (presumably RNA polymerase III) (21, 23). Only the correct strand of the DNA was transcribed and the molecule is reinitiated continually *in vitro* and terminated correctly. 4.5S RNA was also faithfully transcribed, as evidenced by gel electrophoresis.

The relative amounts of 5S and 4.5S RNA synthesized by the chromatin (0.6–1% 5 S, 3–10% 4.5 S) are similar to those made in isolated nuclei (0.25–0.5% 5 S, 2–5% 4.5 S) and *in vivo* (0.89–1.0% 5 S, 4–8% 4.5 S). These genes represent only a small portion of the DNA in the nucleus [0.001% 5 S and 0.005% 4 S (manuscript in preparation)], and yet they account for nearly 1000 times that much RNA. The 45S ribosomal RNA precursor accounts for 40% of the RNA synthesized *in vivo* and *in vitro* (12). The isolated nuclei and chromatin are also similar with respect to α-amanitin sensitivity and hybridization to ribosomal DNA (Marzluff and Huang, unpublished results). Hence, these genes which account for only about 0.04% of the mouse DNA (manuscript in preparation), represent nearly 1000 times that amount of the RNA product. The fact that the maintenance of this dramatic template restriction is continued *in vitro* is striking, particularly in the case of 5S and 4.5S RNA where complete molecules are synthesized. Since the [γ-^{32}P]GTP was only incorporated into low-molecular-weight 4–8S RNA (Fig. 4A), much, if not all, of the 18S and 28S ribosomal RNA synthesis represents elongation of chains initiated *in vivo*. In systems composed of chromatin and added purified RNA polymerase (7, 8) template restriction is not observed, i.e., 40% of the RNA sequences are not rRNA; 1% are not 5S RNA but rather these species are represented more in proportion to their occurrence in DNA (0.01–0.03% in 5 S and <1% is 28 S and 18 S). In addition, in this type of *in vitro* system, correct strand selection is not observed and also "spacer" sequences which are not transcribed *in vivo* are transcribed *in vitro* (7, 8).

5S RNA is transcribed continually from the chromatin template. The total number of 5S RNA molecules synthesized was about 500 to 2000 molecules per haploid amount of DNA, whereas there are about 500 copies of the 5S gene in the mouse genome (manuscript in preparation). Thus, there is net synthesis of 5S *in vitro*. Whether this represents continual release of polymerase and rebinding to the correct site on the DNA is not clear. Possibly multiple polymerases had been bound previously to the correct sites *in vivo*. It is also unclear how many of the 5S genes are active.

We do not know the basis for the relatively high activity of these genes. The isolated polymerases that use chromatin as a template behave essentially identically to bacterial polymerase and do not show this extensive restriction. It is likely that the structure of the genes in chromatin and their interaction with factors other than polymerases result in their great activity *in vitro*. This structure may be very labile and is easily destroyed during routine methods of chromatin preparation.

This work was supported in part by Grants CA 13953 and IROHD 08185 from the National Institutes of Health.

1. Axel, R., Cedar, H. & Felsenfeld, G. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2029–2032.
2. Gilmour, R. S. & Paul, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3440–3442.
3. Steggles, A. W., Wilson, G. W., Kantor, J. A., Picciano, D. J., Falvey, A. K. & Anderson, W. F. (1973) *Proc. Nat. Acad. Sci. USA* **71**, 1219–1223.
4. Astrin, S. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2304–2308.
5. Shih, T. Y., Khoury, G. & Martin, M. A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3506–3510.
6. Jacquet, M., Groner, Y., Monroy, G. & Hurwitz, J. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3045–3049.
7. Reeder, R. H. (1973) *J. Mol. Biol.* **80**, 229–241.
8. Honjo, T. & Reeder, R. H. (1974) *Biochemistry* **13**, 1896–1899.
9. Marzluff, W. F., Murphy, E. C. & Huang, R. C. C. (1973) *Biochemistry* **12**, 3440–3446.
10. Marzluff, W. F., Murphy, E. C. & Huang, R. C. C. (1974) *Biochemistry* **13**, 3689–3696.
11. Zylber, E. & Penman, S. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2861–2865.
12. Reeder, R. H. & Roeder, R. G. (1972) *J. Mol. Biol.*, **67**, 433–441.
13. Price, R. & Penman, S. (1972) *J. Virol.* **9**, 621–626.
14. Price, R. & Penman, S. (1972) *J. Mol. Biol.* **70**, 435–450.
15. Murphy, E. C., Hull, S. H., Shepherd, J. H. & Weiser, A. S. (1973) *Biochemistry* **12**, 3843–3853.
16. Brown, D. D., Wensink, P. & Jordan, E. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 3175–3179.
17. Glynn, I. M. & Chappell, J. B. (1964) *Biochem. J.* **90**, 147–149.
18. Cashel, M. & Kalbacher, B. (1970) *J. Biol. Chem.* **245**, 2309–2318.
19. Hyman, R. & Davidson, N. (1970) *J. Mol. Biol.*, **50**, 421–438.
20. Cedar, H. & Felsenfeld, G. (1973) *J. Mol. Biol.* **77**, 237–254.
21. Weinmann, R. & Roeder, R. G. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1790–1794.
22. Williamson, R. & Brownlee, G. (1969) *FEBS Lett.* **3**, 306–308.
23. Roeder, R. G. (1974) *J. Biol. Chem.*, **249**, 249–256.
24. Weinberg, R. A. & Penman, S. (1968) *J. Mol. Biol.* **38**, 289–304.