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Chromatin as a Template for RNA Synthesis *In Vitro*

(RNA polymerase/polyanions)

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ABSTRACT RNA transcribed *in vitro* from myeloblast chromatin by exogenously added RNA polymerase B predominantly consists of short chains that remain in hybrid structure with the template; the remainder of the product is free RNA of heterogeneous size. Addition of polyanions during synthesis caused an increase in the size and amount of free RNA with a concomitant decrease in the proportion of small RNA. The large molecular weight RNA is derived from the short RNA chains, which are synthesized *de novo* during the reaction *in vitro*. The effect of polyanions on the size and nature of the product may be related to structural changes induced in the template rather than to an inhibition of nuclease activity.

Cells infected with RNA tumor viruses contain virus-specific information in the form of DNA in their genome (1); transcription of this (proviral) DNA is thought to be an obligatory step in viral replication. Since progeny RNA is probably synthesized by host nucleoplasmic RNA polymerase (2, 3) and viral RNA in transcript products can be detected with complementary probe DNA, these cells offer a system for studying the expression of specific genes in eukaryotes. Recently, we reported the presence of virus-specific RNA in products synthesized *in vitro* from myeloblast chromatin by exogenously added eukaryotic RNA polymerase B (2). This chromatin was isolated from myeloblasts of chickens infected with avian myeloblastosis virus (AMV). The proportion of virus-specific sequences in transcript products (1–2%) was approximately the same as that in isolated infected cells (4) and that synthesized in isolated myeloblast nuclei (2). Since the amount of viral RNA in the product was 10^2- to 10^3-fold higher than expected from random transcription, it appeared that the RNA synthesizing system *in vitro* maintained some of the specificity exhibited during transcription *in vivo*. For this reason, we have examined some properties of chromatin-directed RNA synthesis *in vitro*. In this communication we present studies on the rate of chromatin transcription, the structure and size of RNA products formed, and the effect of polyanions on RNA synthesis. The results of these studies are consistent with the suggestion that gene activation in eukaryotes may be caused by an unwinding of chromatin DNA (5).

MATERIALS AND METHODS

Cells and Enzymes. Myeloblasts from AMV infected chickens were generously supplied by Dr. J. W. Beard, Life Science Research Laboratories, St. Petersburg, Fla. HeLa cells and Friend leukemia cells 745A clone 19 were provided by Dr. J. Darnell, Rockefeller University, and Dr. P. Marks, College of Physicians and Surgeons at Columbia University, respectively. *Neurospora* nuclease was purified by the method of Rabin and Fraser (6). RNase H from *Escherichia coli* (7) was purified by Dr. I. Berkower of this department. DNA-dependent RNA polymerase B was purified from calf thymus as described (2); *E. coli* RNA polymerase (holoenzyme) (8) was provided by Dr. L. Yarbrough, of this department.

Isolation of Chromatin. The procedure for the isolation of chromatin from AMV infected chicken myeloblasts was as described (2). Chromatin was isolated from HeLa cells and Friend leukemia cells by the same procedure, except that EDTA (10 mM) was included in the Tris·HCl washes.

Chromatin-Dependent RNA Synthesis. Reaction mixtures (0.05 ml) containing 20 mM Tris·HCl (pH 8.0), 10 mM dithioerythritol, 2 mM $MnCl_2$, 0.6 mM each of ATP, GTP, and CTP, 0.1 mM [^{3}H]UTP or [α-^{32}P]UTP (1 to 2 $\times$ 10^3 cpm/pmol), 100 mM $(NH_4)_2SO_4$, 5–20 μg of chromatin, and 3–6 μg of calf thymus RNA polymerase B or 1–2 μg of *E. coli* RNA polymerase (holoenzyme) were incubated at 37° as indicated in individual experiments. Reactions were stopped by addition of 0.1 ml of 0.1 M sodium pyrophosphate and 50 μg of salmon sperm DNA. Nucleic acids were precipitated with 2 ml of 10% trichloroacetic acid and collected by filtration on Whatman GF/C discs. After three washes with 5% trichloroacetic acid and one wash with 95% ethanol, the filters were dried and radioactivity was determined in a scintillation spectrometer.

Sedimentation Analysis in Sucrose Gradients of RNA Synthesized In Vitro. The synthesis of RNA was as described above; EDTA (50 mM) and sodium dodecyl sulfate (0.1%) were added to stop the reaction. After protein was removed as a protein–sodium dodecyl sulfate complex by centrifugation, samples were incubated at 65° for 10 min in the presence of 6% formaldehyde and 0.1 M sodium phosphate (pH 7.0) and then layered onto 4.8 ml of a 10–30% (w/v) sucrose gradient containing 50 mM Tris·HCl (pH 8.0), 100 mM NaCl, 3% formaldehyde, 1 mM EDTA, and 0.1% sodium dodecyl sulfate. The gradients were centrifuged for 150 min at 49,000 rpm at 24° in an SW 50.1 rotor. Fractions (20 drops) were collected and acid-insoluble radioactivity was determined. Ribosomal RNA markers were run in parallel gradients.

Analysis of the In Vitro Products by Isopycnic Centrifugation in Cs_2SO_4. Deproteinized reaction mixtures (see above) were diluted with 150 mM NaCl, 10 mM sodium phosphate (pH 7.0), 5 mM EDTA, and 0.025% sarkosyl, and Cs_2SO_4 was added to a final density of 1.550 g/ml. After samples were centrifuged 60–70 hr at 30,000 rpm at 25° in an SW 50.1 rotor, 20-drop fractions were collected from a hole pierced in

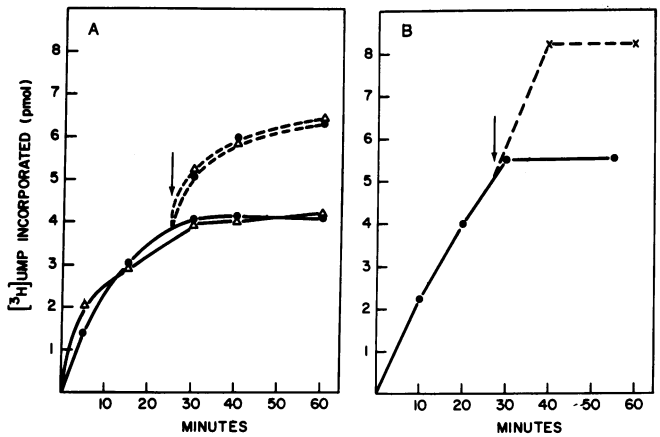

FIG. 1. Rate of RNA synthesis from chromatin and the effect of heparin. (A) Duplicate reaction mixtures (0.15 ml) containing 15 μg of RNA polymerase B and (●——●) 11 μg of chromatin or (△——△) 22 μg of chromatin were incubated at 37°. Additional enzyme (8 μg) was added to one sample at 25 min. At the indicated times, 20-μl aliquots were removed for the determination of ^3H incorporated into acid-precipitable material. (●- - -●) Control; (△ - - -△) additional enzyme. (B) Duplicate reaction mixtures (0.1 ml) containing 10.5 μg of chromatin and 20 μg of RNA polymerase B were incubated as described in panel A. Heparin (2.5 mg/ml) was added to one sample at 27 min. At indicated times, 20-μl aliquots were removed for the determination of [^3H]UTP incorporation into acid-precipitable material. ●, Control; ×, plus heparin.

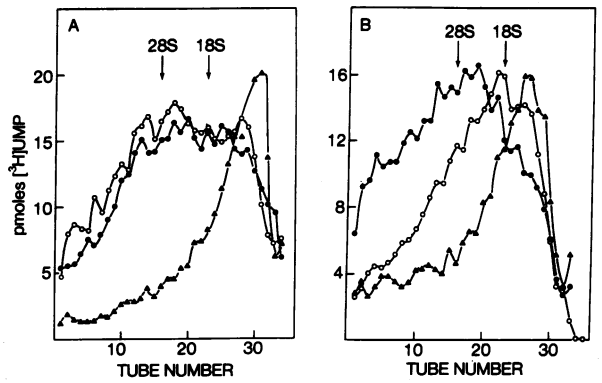

FIG. 2. Sedimentation profile of products transcribed from chromatin in the presence and absence of heparin and poly(rI). (A) RNA synthesis was carried out as described in *Materials and Methods* except for the following: the reaction volume was 0.2 ml and contained 32 μg of chromatin and 40 μg of RNA polymerase B. Reaction mixtures lacking [^3H]UTP (1.5×10^3 cpm/pmol) were incubated for 10 min at 37°, then completed by addition of this nucleotide and incubated for an additional 25 min at 37°. Where indicated, heparin (2 mg/ml) and poly(rI) (0.16 mg/ml) were added 5 min after [^3H]UTP addition. In all cases, reactions were stopped with EDTA and sodium dodecyl sulfate, and the sedimentation profile was determined as described in *Materials and Methods*. ▲, No additions; O, plus heparin; ●, plus poly(rI). (B) The same conditions were used as in panel A except that 2 μg of *E. coli* RNA polymerase replaced RNA polymerase B. ▲, No additions; O, plus poly(rI); ●, plus heparin.

the bottom of the tube. The density of each fraction was determined by measuring the refractive index, and acid-precipitable radioactivity in each fraction was measured.

Nuclease Treatment. Digestion of the samples with RNase H and *Neurospora* nuclease was as described (2). Proteins were determined spectrophotometrically (9), while myeloblast DNA was isolated by the procedure of Gross-Bellard *et al.* (10).

RESULTS

The Kinetics of RNA Synthesis from Myeloblast Chromatin by RNA Polymerase B: the Effect of Heparin on the Kinetics. The rate of RNA synthesis in the presence of excess chromatin and limiting amounts of enzyme declined with time until, at 30 min, further [^3H]UTP incorporation was negligible (Fig. 1)*. At this time, further RNA synthesis was obtained by adding either more enzyme (Fig. 1A) or heparin (Fig. 1B). Heparin is a polyanion that can have multiple effects on the transcriptional system. It reportedly prevents reinitiation of RNA synthesis by DNA-dependent RNA polymerases, inhibits nucleases, and causes the release of certain chromatin-bound proteins (11–13). However, the stimulatory effect of heparin observed in Fig. 1 apparently is unrelated to its inhibitory effect on nucleases since RNA synthesized *in vitro* in the absence of heparin was not degraded under the conditions used (see below).

Size Distribution of the Products Transcribed from Chromatin: the Effect of Polyanions on Product Size. The size of RNA transcribed *in vitro* from myeloblast chromatin by RNA polymerase B was determined after denaturation in formaldehyde followed by sucrose gradient centrifugation. Although some large-molecular-weight RNA was present (Fig. 2A), the majority of the products sedimented as 5–7S material. The addition of heparin or poly(rI) [a polyanion that also prevents reinitiation of RNA synthesis (18)] during the synthetic period not only stimulated RNA synthesis, but also caused a significant increase in the synthesis of large molecular weight RNA†.

These polyanions had similar effects when chromatin was transcribed by *E. coli* RNA polymerase (Fig. 2B), although the products synthesized in the presence of poly(rI) were smaller than those obtained with heparin. It has been reported that the size of RNA products formed from hen oviduct chromatin was increased in the presence of heparin (13). However, the possibility that heparin affected the product size by inhibitory RNase was not excluded in those studies. For this reason the stability of RNA products formed in three identical reaction mixtures containing myeloblast chromatin and RNA polymerase B was examined. After synthesis had progressed for 15 min under conditions described in *Materials and Methods*, α-amanitin (20 μg/ml) was added to stop further RNA synthesis. A control sample was removed and two remaining samples were incubated for an additional 30 min at 37° in the presence or absence of heparin. The sedimentation profiles of these products were identical to that of the control,

* Under similar conditions, RNA synthesis from single-stranded phage DNA (T7 or φX174) was linear for at least 60 min.

† Similar sedimentation profiles were obtained when chromatin prepared from nuclei of HeLa cells or Friend leukemia cells were transcribed by RNA polymerase B in the presence and absence of heparin.

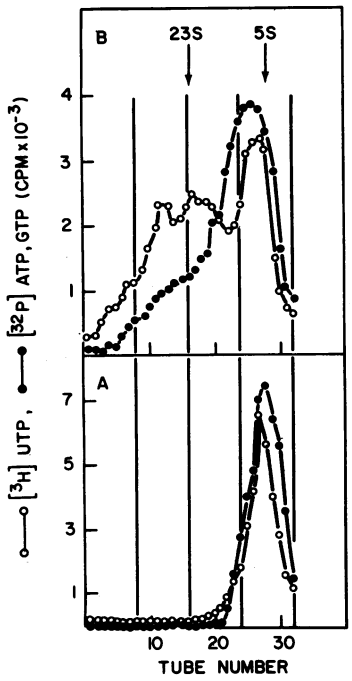

FIG. 3. Sedimentation profiles of chromatin transcripts pulse-labeled with [^{3}H]UTP, [γ-^{32}P]ATP, and [γ-^{32}P]GTP and then chased with unlabeled UTP. Duplicate reaction mixtures similar to those described in *Materials and Methods* but containing, in a total volume of 0.1 ml, 10 μg of RNA polymerase B, 24 μg of chromatin, unlabeled CTP, and 0.06 mM each of [γ-^{32}P]ATP and [γ-^{32}P]GTP (both 2 $\times$ 10^4 cpm/pmol) were preincubated for 10 min at 37°. Synthesis was started in the presence of heparin (2 mg/ml) by the addition of [^{3}H]UTP (900 cpm/pmol). In other experiments (not presented above) it was shown that the presence of heparin blocked incorporation of [γ-^{32}P]ATP and [γ-^{32}P]GTP. O, [^{3}H]UTP; ●, [γ-^{32}P]ATP and [γ-^{32}P]GTP. (A) After 30 sec, the reaction was stopped with EDTA and sodium dodecyl sulfate, unlabeled ATP and GTP (2.5 μmol each) were added, and the sample was dialyzed against a solution containing 10 mM Tris·HCl (pH 7.5) and 0.1% sodium dodecyl sulfate. After the products were heated in 6% formaldehyde, they were sedimented in sucrose gradients as described. (B) Unlabeled UTP was added at 30 sec to yield a final concentration of 20 mM and incubation at 37° was continued for an additional 5 min.

indicating that RNA degradation by RNase did not occur‡. These results also indicate that heparin affects the size distribution of the products only during RNA synthesis.

Evidence for De Novo Synthesis of RNA In Vitro. The large-molecular-weight products formed during chromatin transcription *in vitro* could result either from *de novo* synthesis of RNA chains or from the elongation of preexisting RNA molecules. Evidence that large-molecular-weight RNA synthesized *in vitro* was derived from chains initiated during the reaction was obtained (Fig. 3). The size of RNA products formed after pulse labeling with [^{3}H]UTP, [γ-^{32}P]ATP, and [γ-^{32}P]GTP followed by a chase period with unlabeled UTP in the presence of heparin was examined. Prior to the chase all RNA products sedimented as 5–7S material and contained both radioactive labels (Fig. 3A); after the chase period with unlabeled UTP the doubly labeled product increased in size, with a concomitant decrease in material sedimenting in the 5–7S region (Fig. 3B). The size of the RNA products determined by sedimentation (Fig. 3B) and by average chain length measurements (ratio of ^{3}H:^{32}P incorporated) were approximately the same; these results indicate that the majority of RNA produced was synthesized *de novo*. Moreover, after alkaline hydrolysis all [^{32}P]products migrated as nucleoside tetraphosphates when subjected to electrophoresis on paper.

‡ No detectable DNase activity was observed in mixtures containing myeloblast chromatin and RNA polymerase B. These preparations were incubated in a standard RNA synthesis mixture with unlabeled UTP and labeled ϕX RF1 DNA. No detectable decrease in the sedimentation profile of ϕX RF1 was observed in alkaline sucrose gradients (14).

TABLE 1. *Size of RNA transcripts from various DNA templates and chromatin*

Template used	Size of RNA products formed with: RNA polymerase B	Size of RNA products formed with: *E. coli* RNA polymerase
Single-stranded DNA		
ϕX174 viral	15–25S	6–10S
T7 (denatured)	20–30S	5–10S
Native DNA		
ϕX174 RF1	$\leq$7S	16–30S
T7	<5S	20–30S
Chromatin (myeloblast, HeLa, Friend)	7–50S	7–50S
Double-stranded DNA from chromatin (myeloblast, HeLa)	$\leq$5S	7–40S

Reaction mixtures for RNA synthesis (0.05 ml) described in *Materials and Methods* were incubated for 30 min. Where indicated, DNA from ϕX174 (3.5 μg), ϕX174 RF1 (1.2 μg), native or denatured T7 (1.2 μg), chromatin (5 μg), or DNA isolated from chromatin (10) (4.5 μg) was used with 10 μg of RNA polymerase B or 1 μg of *E. coli* RNA polymerase (holoenzyme). Formaldehyde treatment of products and sedimentation in sucrose gradients containing formaldehyde were performed as described in *Materials and Methods*. S values were calculated from *E. coli* or HeLa cell ribosomal RNA markers, which were cosedimented in separate tubes.

Size of RNA Products Transcribed by RNA Polymerase B from Single- and Double-Stranded DNA. RNA polymerase B uses single-stranded DNA as a template more efficiently than native DNA (15–17). To determine whether the nature of the DNA also affects the size of RNA products, DNA isolated from a variety of sources, including phages, were used as template. The size distributions of RNA products formed from single-stranded and double-stranded DNA were examined (Table 1). In contrast to the large products synthesized by *E. coli* RNA polymerase from native DNAs, products formed from these DNAs by RNA polymerase B were small. However, relatively large transcripts were obtained when the eukaryotic enzyme transcribed single-stranded DNA. Neither heparin nor poly(rI) affected the size distribution of the products during transcription of DNA. It is of interest that RNA polymerase B synthesized only small products when native DNA from isolated chromatin served as template, whereas it synthesized both large and small molecular weight material from chromatin (Table 1).

TABLE 2. *RNase H and Neurospora nuclease sensitivity of products transcribed from chromatin in the presence of poly(rI)*

Condition of RNA synthesis	Synthesized	Degraded by *Neurospora* nuclease	Degraded by RNase H
(a) Poly(rI) added at 5 min	18.5	12.0 (65)	3.0 (16)
(b) Poly(rI) added at end of incubation	11.0	2.1 (19)	6.3 (57)

Reaction mixtures for RNA synthesis (0.1 ml), containing 16 μg of chromatin and 10 μg of RNA polymerase B, were incubated for 30 min. Poly(rI) (8 μg) was added at 5 min (a) or at the end of the incubation period (total of 30 min) (b). To stop reactions, 2 μg of α-amanitin was added at 30 min to both (a) and (b), and the amount of RNA degraded by RNase H or *Neurospora* nuclease was determined on aliquots from each reaction mixture. Values are in pmol. The numbers in parentheses represent the percentage of RNA degraded after the indicated treatments.

Analysis of Product Structures by Isopycnic Banding in Cs_2SO_4: the Effect of Polyanions on Product Structure. During RNA synthesis newly synthesized RNA is hydrogen-bonded with the DNA template; this RNA is displaced by the complementary strand when DNA is in duplex structures, but remains in hybrid structures with single-stranded DNA templates. Thus, analysis of the nature of the RNA product could provide information concerning the DNA in chromatin that serves as template during transcription. The results of this type of analysis are presented in Fig. 4. Reaction mixtures containing synthesized RNA (after deproteinization) were subjected to isopycnic centrifugation in Cs_2SO_4. Approximately 70% of the [^{32}P]RNA banded at densities characteristic of RNA·DNA hybrid structures and of DNA (Fig. 4A); the latter would occur if small products were hydrogen-bonded with large fragments of DNA. The identity of the hybrid structures was confirmed in the following manner. Treatment of products with pancreatic DNase prior to centrifugation caused the radioactive material to shift into RNA regions without loss of radioactivity (Fig. 4B). Treatment with *Neurospora* nuclease resulted in a disappearance of single-stranded RNA (RNA region), and a shift of material from the DNA region to the density of RNA·DNA hybrid structures (Fig. 4C). On the other hand, digestion by RNase H (Fig. 4D) caused the disappearance of 70–80% of the total product that had originally banded as hybrid structures; the remaining product banded at the density of free RNA. Thus, these results suggest that approximately 70% of the product synthesized by RNA polymerase B is transcribed from single-stranded regions of chromatin DNA.

Transcription of chromatin in the presence of heparin and poly(rI) caused significant changes in the proportion of products that remained in hybrid structure. The product formed in the presence of poly(rI) was less sensitive to the action of RNase H than products formed in its absence (Table 2). Concomitant with this, there was an increase in the susceptibility of the RNA products formed in the absence of poly(rI) to *Neurospora* nuclease. Heparin had parallel effects on the amount of product present as free RNA compared to that present in RNA·DNA hybrid structures. The products synthesized in the presence of heparin (Fig. 5A) contained less material that banded at the density of DNA or RNA·DNA

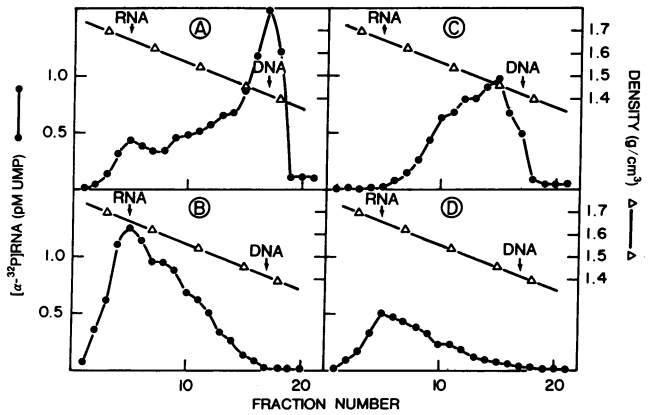

FIG. 4. Analysis of products transcribed from chromatin. RNA was synthesized in 0.25-ml reaction mixture (see Table 3) containing chromatin (50 μg) and RNA polymerase B (20 μg of protein). The product was extracted with phenol and filtered through a Sephadex G-50 column. Aliquots were incubated for 30 min at 37° with the following enzymes: A, none; B, DNase (50 μg/ml); C, *Neurospora* nuclease (1.2 units/ml); D, RNase H (40 units/ml). All fractions were analyzed by isopycnic centrifugation in Cs_2SO_4 as described in *Materials and Methods*.

hybrid structures than products formed in its absence (Fig. 5D); furthermore, heparin caused a large increase in the proportion of free RNA. To ensure that heparin had not interfered with the banding of products during isopycnic centrifugation in Cs_2SO_4, material in the RNA and DNA regions of the gradient was examined for RNase H sensitivity. Table 3 shows that the portion of the product in the DNA region was 85% sensitive to RNase H, while material in the RNA region of the gradient was not degraded by RNase H but was sensitive to *Neurospora* nuclease. Since polyanions caused an increase in the size of the RNA transcribed from chromatin, the effects of heparin on the size of the products present as free RNA and in RNA·DNA hybrid structures were examined. As seen in Fig. 5B and E, RNA in hybrid structure sedimented as 5–7S material in sucrose gradients whether heparin was present or absent during synthesis. However, the portion of the product that consisted of free RNA had a heterogeneous size distribution (Fig. 5C and F) and its size and amount significantly increased during synthesis in the presence of heparin (Fig. 5). Thus, in the presence of polyanions transcription occurs predominantly from regions of chromatin DNA that retain some of a double-stranded character since the product is displaced (presumably) by the complementary DNA strand. Moreover, transcription of these regions yields large-molecular-weight products.

DISCUSSION

Large-molecular-weight RNA products are formed by RNA polymerase B in reactions with single-stranded DNA template; the RNA is hydrogen-bonded to the DNA. In contrast, with native DNA as template, small-molecular-weight products are formed. In addition, native DNA acts as a poor template, while more extensive RNA is formed with single-stranded DNA.

The products synthesized *in vitro* from chromatin by RNA polymerase B were analyzed with respect to structure and size to provide information concerning the nature of the DNA that served as template. The majority of newly synthesized RNA was small molecular weight and remained in duplex structure

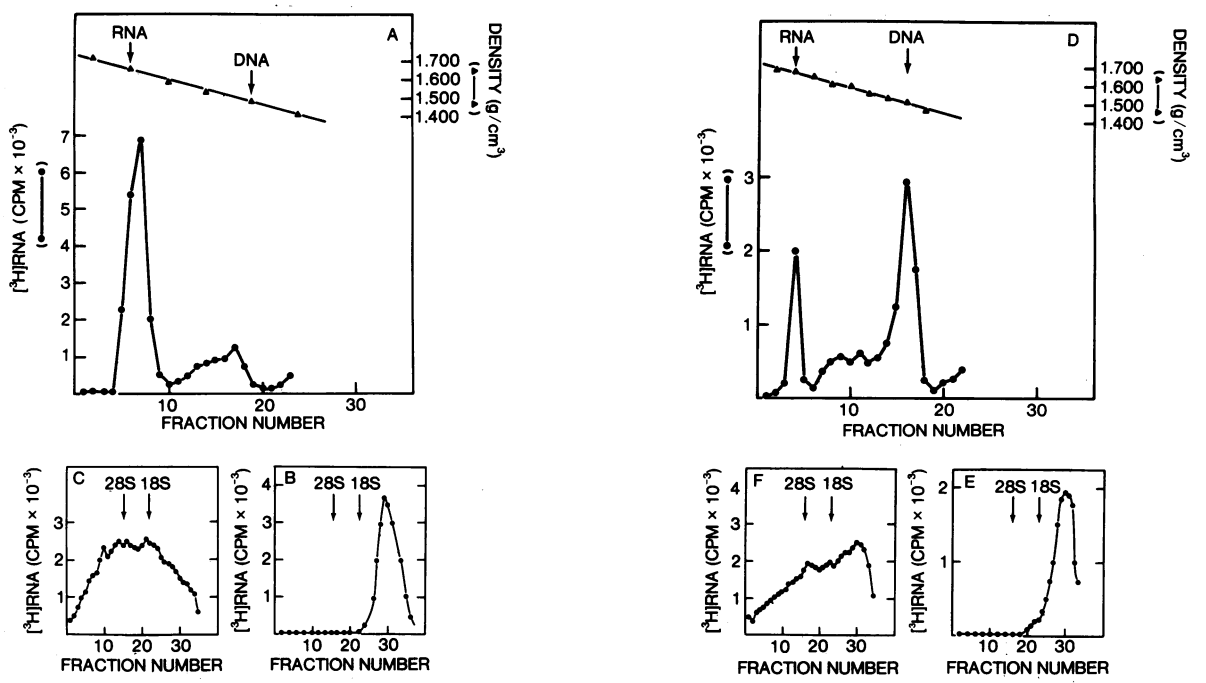

FIG. 5. Analysis of products transcribed from chromatin in the presence or absence of heparin. (A and D) *Isopycnic centrifugation in Cs_2SO_4.* Reaction conditions were as described in the legend of Fig. 2 except that [³H]UTP was 14,000 cpm/pmol and incubation time after addition of labeled substrate was 30 min. (A) Heparin (2.5 mg/ml) was added 5 min after addition of [³H]UTP. (D) Incubation in the absence of heparin. Reactions were stopped by addition of EDTA and sodium dodecyl sulfate; the final concentrations were 50 mM and 0.2%, respectively. Isopycnic centrifugation in Cs_2SO_4 was carried out as described in *Materials and Methods*. The acid-precipitable radioactivity in 25-μl aliquots of each fraction is presented above. (B and E) *Sedimentation in sucrose gradients of material that banded in Cs_2SO_4 as RNA·DNA hybrid structures (DNA region).* Material that banded in Cs_2SO_4 at densities of 1.46–1.53 g/cm³ was pooled and dialyzed for 16 hr against medium containing 1 mM Tris·HCl (pH 7.5), 0.1% sodium dodecyl sulfate. (B) Fractions 16 to 18 from Cs_2SO_4 gradient described in panel A. (E) Fractions 15 to 18 from Cs_2SO_4 gradient described in panel D. After the products were heated for 10 min at 65° in the presence of 6% formaldehyde, samples were sedimented in 10–30% sucrose gradients as described in *Materials and Methods*. (C and F) *Sedimentation in sucrose gradients of material that banded at the density of RNA.* Portions of the products that banded in Cs_2SO_4 at densities of 1.63–1.68 g/cm³ were pooled. (C) Fractions 5 through 8 from Cs_2SO_4 gradient of panel A. (F) Fraction 4 from Cs_2SO_4 gradient of panel D. After dialysis, samples were heated in the presence of formaldehyde and sedimented in sucrose gradients as described above.

with the template. The remainder of the product was free RNA of heterogeneous size. In the presence of polyanions [heparin or poly(rI)] that stimulated net RNA synthesis, the proportion and size of the free RNA increased. It has been reported (13) that the amount and size of RNA products increased when transcription of hen oviduct chromatin was carried out in the presence of heparin. These effects were attributed to the inhibitory action of the polyanion on nucleases contaminating the preparations of hen oviduct chromatin. The increase in RNA synthesis and product size observed in the present studies probably resulted from a different effect since the chromatin preparations used appeared free of RNase activity. The observations that transcription of DNA was unaffected by polyanions, and that the size of RNA products transcribed from chromatin by *E. coli* RNA polymerase was increased by the presence of polyanions, suggest that these compounds may affect chromatin transcription by causing structural modifications of the template.

TABLE 3. *Sensitivity of RNA isolated from different regions of Cs_2SO_4 gradient to RNase H and Neurospora nuclease*

Density region of Cs_2SO_4 gradient examined	RNA		
	Assayed	Degraded by RNase H	Degraded by RNase H + *Neurospora* nuclease
DNA	1.92	1.63 (85)	1.87 (98)
RNA	4.20	0.18 (4.3)	3.98 (95)

Portions of the product transcribed in the presence of heparin which banded in Cs_2SO_4 (Fig. 5A) at densities 1.63–1.68 g/ml (RNA region) and 1.46–1.53 g/ml (DNA region) were pooled, dialyzed for 16 hr against 10 mM Tris·HCl (pH 7.5), and incubated with RNase H and RNase H plus *Neurospora* nuclease as described in *Materials and Methods*. Values are in pmol. The numbers in parentheses represent the percentage of RNA remaining after the indicated treatments.

During transcription, newly synthesized RNA is hydrogen-bonded with the DNA template. This RNA is readily displaced when the DNA is in duplex structure, but remains in hybrid structure with a single-stranded DNA template. It, therefore, appears that the majority of the products synthesized in the *absence* of polyanions were transcribed from single-stranded regions of the DNA in chromatin§. Furthermore,

§ Further support for this was obtained; chromatin-directed RNA synthesis was inhibited by *E. coli* DNA binding protein (which specifically binds to single-stranded DNA) (20) and by pretreatment of the template with *Neurospora* nuclease.

initiation probably occurred in these single-stranded regions since RNA polymerase B binds preferentially to single-stranded templates (19). The relatively small size of RNA products in hybrid structures could reflect the extent of the single-stranded region available to the enzyme. Studies with different templates have shown that RNA polymerase B synthesizes relatively long RNA chains from single-stranded templates. The results of pulse-chase experiments indicated that large-molecular-weight products were derived from newly initiated small RNA chains. Since this RNA was displaced from the template, transcription probably continued into regions of chromatin DNA that retained double-stranded characteristics. However, the structure of the DNA in *these* regions of the template must have been partially denatured since only short RNA chains are transcribed from isolated native DNA. It is likely that these regions are limited in the absence of polyanions, as indicated by the finding that a relatively small portion of the products is present as large-molecular-weight free RNA. Polyanions, by causing a release of some chromatin-bound proteins (see ref. 13), could affect transcription by increasing the availability of "partially denatured" regions of chromatin DNA. The effects of polyanions on the yield of transcription are consistent with this hypothesis. Since these compounds inhibit reinitiation, the resumption of RNA synthesis caused by heparin [and poly(rI)] must reflect an extension of RNA chains still containing polymerase at the 3'-OH end into previously inaccessible regions of chromatin DNA.

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Note Added in Proof. Howk *et al.* (21) have recently made similar observations about the nature of the chromatin transcript products, using chromatin and RNA polymerase II isolated from mouse cells.

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