Proc. Nat. Acad. Sci. USA
Vol. 73, No. 4, pp. 1121–1125, April 1976
Biochemistry

Double-stranded RNA in chromatin transcripts formed by exogenous RNA polymerase

(endoribonuclease III/ribonuclease resistance/intramolecular base pairing)

ETIENNE PAYS

Laboratoire de Cytologie et d'Embryologie Moléculaires, Université Libre de Bruxelles, 67, rue des Chevaux, B1640 Rhode-Saint Genèse, Belgium

Communicated by Jean Brachet, January 27, 1976

ABSTRACT RNA transcribed *in vitro* at low ionic strength, from either rat liver chromatin or DNA, contains a significant amount of structure resistant to RNase in high salt buffer. This is observed with rat liver (form B polymerase) as well as with *Escherichia coli* RNA polymerase (RNA nucleotidyltransferase; nucleoside triphosphate:RNA nucleotidyltransferase; EC 2.7.7.6). Treatment with RNases specific for either double-stranded or hybrid RNA indicates that resistance to RNase is due to the presence of double-stranded RNA sequences. Denaturation kinetics in the presence or absence of RNase suggest that these sequences are formed by intramolecular base pairing. Their mean length is about 20 to 30 nucleotides, but 15–20% are more than 100 nucleotides long. They contain 60–65% G·C base pairs. The proportion of double-stranded segments is higher in chromatin transcripts than in DNA-templated RNA, and is higher with homologous RNA polymerase than with the bacterial enzyme. On the other hand, chromatin endogenous RNA polymerase, which is unable to initiate transcription, does not synthesize double-stranded RNA. The problem of the location of these sequences is discussed; preliminary results suggest that the 5′ end of the RNA transcripts could be enriched in complementary sequences.

RNA synthesized *in vitro* from chromatin by exogenously added RNA polymerase (RNA nucleotidyltransferase; nucleoside triphosphate:RNA nucleotidyltransferase; EC 2.7.7.6) seems to be specific (1–9). Eukaryotic or bacterial RNA polymerases give essentially the same results, although transcription specificity seems to be better with the homologous enzyme (8), and the initiation sites on the DNA differ for the two RNA polymerases (10–12). This may mean that only a few specific sequences are accessible to RNA polymerase in chromatin.

The *in vitro* RNA synthesizing system provides an opportunity for studying the initial products of transcription. We were interested to find out whether middle repetitive sequences were transcribed first for regulatory functions (13), since they appear to be interspersed in the rat genome (14). We have previously shown (unpublished results) that repetitive sequences of liver chromatin transcripts are similar to those from liver nuclear RNA, but different from those of kidney nuclear RNA. In the course of these hybridization experiments, we were brought to examine RNA sequences which were resistant to RNase in high salt without prior denaturation and self-annealing.

MATERIALS AND METHODS

Enzymes. RNase A (EC 3.1.4.22) and RNase T1 (EC 3.1.4.8) were purchased from Sigma. They were heat-treated for 10 min at 95° before use. RNase III (EC 3.1.4.24) and RNase H (EC 3.1.4.34) were kindly provided by Dr. J. L. Darlix (Gif-sur-Yvette). DNase I (EC 3.1.4.5) (RNase-free grade) was from Worthington.

Extractions and Purifications. Chromatin was extracted from purified rat liver nuclei as described in ref. 15, except that Nonidet was added only in the last steps of nuclei purification. On the other hand, mixtures for chromatin extraction always contained 20% (vol/vol) glycerol and 0.1 mM dithiothreitol. Chromatin was not sheared and was not stored longer than 24 hr at 4°.

DNA was purified as described (15), except that phenol extractions were replaced by banding in a CsCl gradient.

RNA polymerases from *Escherichia coli* or rat liver (form B) were extracted and purified as described (15). Their activity is absolutely dependent on the presence of template. When necessary, rat liver RNA polymerase was treated with DNase and re-chromatographed on DEAE-Sephadex, in order to suppress completely the enzyme activity without exogenous template.

***In Vitro* RNA Synthesis.** RNA was synthesized in 5 ml of the following incubation mixture: 5 mM dithiothreitol, 5 mM $(NH_4)_2SO_4$, 2 mM $MnCl_2$, 0.1 mM ATP, 0.07 mM [γ-^{32}P]GTP (3.7 Ci/mmol), 0.016 mM [^{3}H]UTP (6 Ci/mmol), 0.016 mM [^{3}H]CTP (6 Ci/mmol), 50 mM Tris·HCl (pH 7.9), 100 μg of template, and 1 μg of *E. coli* RNA polymerase or 500 units of rat liver RNA polymerase B (see ref. 15). These ionic conditions were found to minimize endogenous RNA polymerase activity (no more than 13% of total incorporation), while allowing optimal transcription by the exogenously added enzyme. At higher ionic strengths, the endogenous RNA polymerase activity of the chromatin is progressively enhanced [with a sharp increase at 0.175 M $(NH_4)_2SO_4$], while exogenous polymerase initiation on DNA is inhibited. It is to be noted here that the incubation mixture contains 5–8% (vol/vol) glycerol, due to the dilution of the media for enzyme and template storage. Synthesis by exogenous rat liver RNA polymerase B is fully α-amanitin (0.1 μg/ml) sensitive; endogenous RNA polymerase activity is 50% inhibited by this drug. Maximal incorporation is attained after 2 hr with exogenous RNA polymerase, while a plateau is reached after 15–30 min by chromatin endogenous polymerase. The RNA remains fully trichloroacetic-acid-precipitable for at least 8 hr in the incubation medium. Per 50 units of RNA polymerase B, 0.04 μg of RNA are synthesized on 10 μg of chromatin DNA; in the conditions we used, the amount of *E. coli* RNA polymerase was adjusted to obtain an equivalent level of transcription. RNA synthesis was stopped by the addition of EDTA (10 mM final; pH 8); proteinase K (Merck) was then added (500 μg/ml) and incubation was continued for 30 min at 37°. The medium was adjusted to 0.1 M NaCl, 2% sodium dodecyl sulfate, and 1 mg/ml of Pronase B (nuclease-free), and incubated for 1 hr at 37°. RNA was then deproteinized by phenol–$CHCl_3$ (1:1)

Abbreviations: dsRNA, double-stranded RNA; hnRNA, heterogeneous nuclear RNA; T_m, melting temperature.

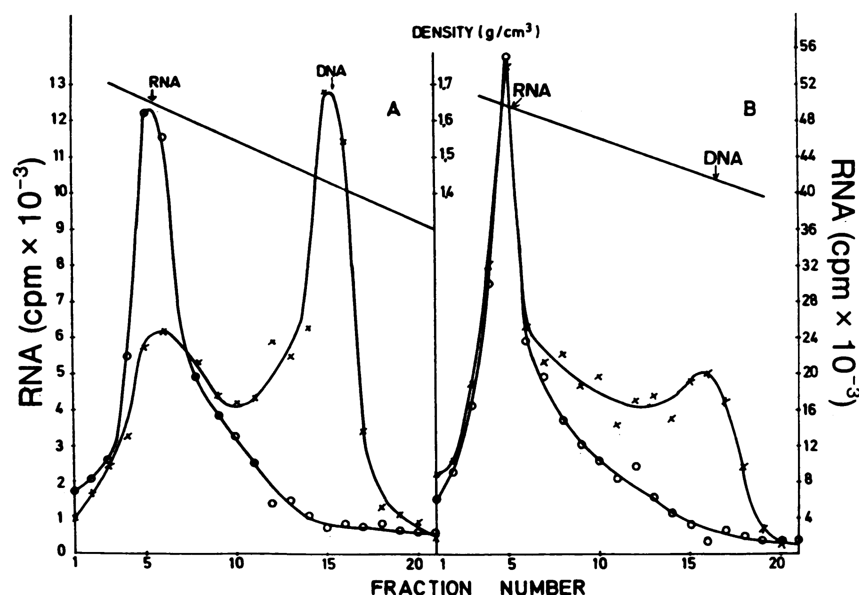

FIG. 1. Analysis of RNA transcripts on Cs_2SO_4 gradients, before and after DNase treatment. RNA was synthesized on DNA by rat liver RNA polymerase B (A) or *E. coli* RNA polymerase (B), and deproteinized as described in *Materials and Methods*. (O): RNA purified by DNase treatment in the usual way; (X): RNA purified without DNase treatment. The markers refer to the position of yeast RNA and rat liver DNA, run separately to calibrate the gradients.

extractions at 25°, and precipitated in ethanol. It was dissolved in a small volume of 10 mM Tris-HCl (pH 8) and passed through a Sephadex G-75 column. The pooled fractions from the excluded peak were heated for 5 min at 70°, adjusted to 10 mM $MgCl_2$, and incubated for three 20 min periods, each with 50 μg/ml of DNase I at 37°, in the presence of 100 μg of yeast RNA. After phenol-$CHCl_3$ extractions, RNA was precipitated in ethanol and passed through a Sephadex G-75 column. The recovery of RNA is estimated to be around 70%; this is due to the elimination of the trailing edge of the excluded peak on the two Sephadex columns (representing very small RNA molecules). RNA synthesized *in vitro* was completely hydrolyzed in 0.3 M KOH within 2 hr at 37°.

Heterogeneous Nuclear RNA (hnRNA) was extracted in the same way from purified rat liver nuclei, 30 min after injection of 2.5 mCi of [^{3}H]orotic acid and 0.5 mCi of [^{3}H]cytidine in the peritoneal cavity of young rats (150 g). Only the RNA present in the aqueous phase of phenol extractions above 60° was recovered. This RNA has a sedimentation constant greater than 23 S.

RNase Digestions. "Usual" RNase digestion refers to an incubation of RNA with 100 μg/ml of RNase A and 50 units/ml of RNase T1 for 2 hr at 37° in 0.5 ml of 0.3 M NaCl, 10 mM EDTA, and 5 mM Tris-HCl (pH 8). Residual RNA was precipitated in 10% trichloroacetic acid with 100 μg of yeast RNA as carrier, and collected by filtration on Whatman GF/C filters.

Incubations with RNase III and RNase H were performed for 2 hr at 37° in 0.5 ml of 0.3 M NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM dithiothreitol, and 40 mM Tris-HCl (pH 7.9). The specificity of these RNases was checked on a [^{3}H]RNA-DNA hybrid obtained by annealing rat DNA with rat liver [^{3}H]RNA at a C_0t value (concentration × incubation time) = 2.94×10^3 (moles of nucleotide/liter) × sec. In the conditions used, no attack on the hybrid by RNase III was detectable, while RNase H was fully effective.

Isopycnic Centrifugation in Cs_2SO_4. RNA was centrifuged with 100 μg of yeast RNA as carrier, following the method of Groner *et al.* (16). Density was determined by measuring the refractive index at 20°. Yeast RNA and rat liver DNA were run separately to calibrate the gradients.

Polyacrylamide Gel Electrophoresis. Electrophoresis of RNA was performed following Loening (17) in 2.7 or 10% acrylamide gels with 0.2% sodium dodecyl sulfate. *E. coli* ribosomal 16S RNA and tRNA were used as markers. Fractions of 2 mm length were dissolved and counted in 5% Soluene (Packard)/Omnifluor.

Base Composition Analysis. RNA was hydrolyzed with 1 mg of yeast RNA in 0.5 M KOH for 15 hr at 30°, then acidified with perchloric acid. After 15 min at 0°, potassium perchlorate was centrifuged, and the neutralized supernatant was eluted on a Dowex AG1X2 column with a linear HCl gradient. CMP and UMP were eluted as the first and last nucleotides, respectively. Acid hydrolysis (in 1 M perchloric acid for 48 hr at 37°) was also performed with identical results.

RESULTS

Analysis on Cs_2SO_4 Gradients. RNA synthesized on either chromatin or DNA by rat liver RNA polymerase B is mostly in an RNA-DNA hybrid structure having the density of DNA, as shown (for a DNA template) by Cs_2SO_4 gradient analysis (Fig. 1A). On the other hand, in conditions allowing the synthesis of about the same amount of RNA by the two RNA polymerases, the majority of both the chromatin and DNA transcripts of *E. coli* RNA polymerase are not hybridized to DNA (Fig. 1B for DNA transcripts). After DNase treatment (see *Materials and Methods*), RNA seems to be free, but a shoulder is observed in the region of intermediate density between RNA and DNA (Fig. 1A–B). This fraction is enriched in RNA which is resistant to RNase digestion at high ionic strength, as seen in Fig. 2. The shift in the mean density from 1.65 g/ml for undigested RNA to 1.62 g/ml after RNase digestion suggests the presence of double-stranded RNA (dsRNA) in the transcripts, since dsRNA has a somewhat lower density than native RNA (18–20). However, a broadening of the profile after RNase digestion indi-

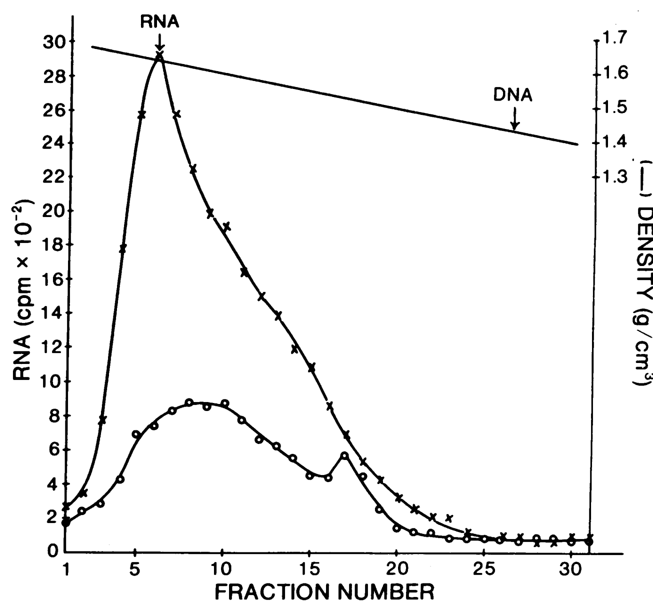

FIG. 2. Analysis of chromatin RNA transcript on Cs_2SO_4 gradient, before and after RNase treatment in 0.3 M NaCl. RNA was synthesized on chromatin by rat liver RNA polymerase B and purified as described in *Materials and Methods*. (×): control RNA; (O): RNA treated with RNase in high salt buffer.

cates that a diffusion of RNA molecules has taken place. This is due to a shortening of RNA length from a mean of about 100 to 200 nucleotides before RNase, to about 20 to 30 nucleotides after digestion, with 15–20% of RNA still above 100 nucleotides long, as revealed by gel electrophoresis (not shown).

Resistance of Transcripts to RNase. We have studied the resistance of our transcripts to RNases A + T1 by comparing it with that of other RNAs (Table 1). It seems that the chromatin transcripts by exogenous polymerase are particularly rich in sequences resistant to RNase in high salt buffer, while RNA synthesized by the endogenous RNA polymerase does not contain resistant structures. Resistance of DNA-templated transcripts is low, being of the same order as that of heterogeneous nuclear RNA, whereas poly(U) is completely degraded under the same conditions. On the other hand, the resistance of all transcripts is lower than 2% in 15 mM NaCl. The percentage of resistance is variable from one experiment to another (depending on the amount of RNA recovered), but the proportions of resistance between these

Table 1. Resistance of different RNAs to RNase in 0.3 M NaCl

	Resistance (%)	
	Exp. 1	Exp. 2
In vitro transcripts from:		
Chromatin + liver polymerase	43.6	24.3
Chromatin + *E. coli* polymerase	24.6	15.0
Chromatin (endogenous polymerase)	0	0
DNA + liver polymerase	3.9	2.7
DNA + *E. coli* polymerase	6.4	4.1
hnRNA from rat liver	5.7	
Poly(U)	0.2	

Incubations were as described in *Materials and Methods*. In each case, resistance in 15 mM NaCl was below 2%. Chromatin and DNA were from rat liver.

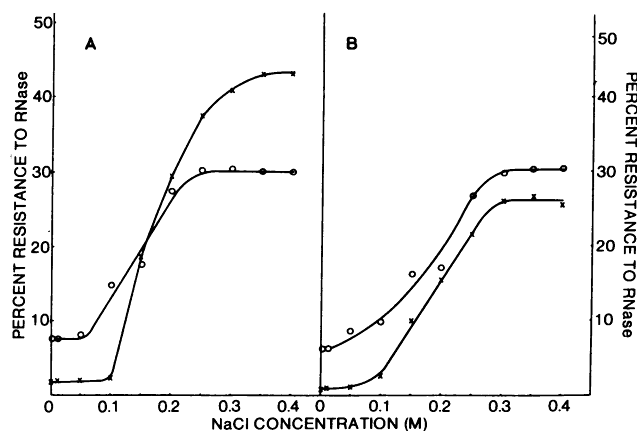

FIG. 3. Effect of ionic strength on resistance of chromatin RNA transcript to RNAse. RNA was synthesized on chromatin by rat liver RNA polymerase B (A) or *E. coli* RNA polymerase (B), then purified as in *Materials and Methods*. It was digested by 100 μg/ml of RNase A and 50 units/ml of RNase T1 in 5 mM Tris·HCl (pH 7.9) containing various NaCl concentrations. RNA was labeled with [γ-^{32}P]GTP, [^{3}H]UTP, and [^{3}H]CTP as described in *Materials and Methods*. (O): percentage of ^{32}P resistance to RNase; (×): percentage of ^{3}H resistance.

different RNAs remain rather constant. A detailed analysis of the ionic strength dependence of this resistance is represented in Fig. 3: chromatin transcripts become stabilized at concentrations of NaCl above 0.1 M at 37° and are optimally protected at 0.3 M NaCl. In high salt, RNase digestion reaches a plateau within 15 min, whereas it continues for a longer period in 0.15 M NaCl (Fig. 4). At the time of maximal hydrolysis in 0.3 M NaCl, new addition of RNase is without effect, while a shift to a higher temperature (23°–37°) leads to an increase in digestion (Fig. 4). This greater susceptibility to digestion at 37° probably reflects denaturation of some resistant structures at this temperature. Therefore, we investigated the effect of thermal denaturation on resistance to RNases A + T1 (Fig. 5A–B). When the enzymes are present during melting, about 20% of resistant RNA undergoes denaturation and digestion at a melting temperature T_m of 43°. On the other hand, adding the enzymes after cooling the samples back to 37° allows these sequences to renature quickly and thus escape digestion; in this case, the RNase-resistant RNA appears to melt with a T_m of 115° in 0.3 M NaCl and 100° in buffer (Fig. 5A). The presence of 40% (vol/vol) formamide lowers the T_m by approximately 15° in high salt and 20° in low salt buffer (Fig. 5B). The denaturation occurs over a very large temperature interval, which could mean that spontaneous renaturation competes with the denaturation process. This interpretation is strongly supported by the fact that denaturation in high salt becomes much more rapid and complete if RNase is present during melting, in conditions unfavorable for spontaneous renaturation of RNA when the samples are cooled back to 0° (Fig. 5).

Additional Evidences for dsRNA in Chromatin Transcripts. We initially observed that DNase I treatment of resistant RNA does not increase its sensitivity to RNase attack. The absence of residual RNA·DNA hybrids in resistant RNA was further confirmed by the results of enzymatic tests with RNase H, specific for RNA·DNA hybrids, and RNase III, specific for dsRNA (Table 2). It appears that RNase H free of contaminating RNase III activity (21) does not degrade RNase-resistant transcripts. Moreover, the ability of RNase

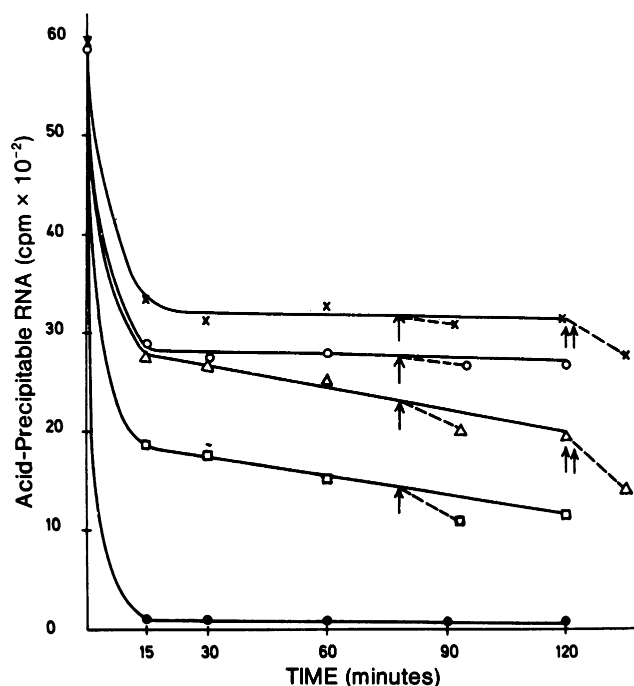

FIG. 4. Kinetics of RNase action on chromatin RNA transcript. RNA was synthesized on chromatin by rat liver RNA polymerase B and purified as described in *Materials and Methods.* It was digested by 100 μg/ml of RNase A and 50 units/ml of RNase T1 in 5 mM Tris·HCl (pH 7.9) containing 0.3 M NaCl (×, ○), 0.15 M NaCl (△, □), or 15 mM NaCl (●). Incubations were performed at 23° (×, △, ●) or 37° (○, □, ●). At the time indicated by a single arrow, the amount of RNase was doubled and incubation was continued for 15 min. At the time indicated by two arrows, the incubation temperature was shifted from 23 to 37° and incubation was continued for 15 min.

III to digest all of the resistant RNA in high salt indicates that it consists of dsRNA.

Base Composition of dsRNA. RNA synthesized on chromatin by rat liver RNA polymerase B was analyzed for its CMP and UMP content. The pyrimidines of the total transcripts were found to contain 51.6–52.7% [^{3}H]CMP and 48.4–47.3% [^{3}H]UMP, whereas the double-stranded structures contained 60.3–65.4% CMP and 39.7–34.6% UMP: these are thus clearly enriched in G·C base pairs.

Location of dsRNA in the Transcripts: Preliminary Results. The initial nucleotide was labeled by adding [γ-^{32}P]GTP in the incubation medium (see *Materials and Methods*). A significant fraction of [^{32}P]GTP is present in RNase-resistant structures (Fig. 3). Thus, at least a fraction of *in vitro* transcribed RNA could contain a double-stranded structure at the beginning of the chain. We have verified that the gamma phosphate does not turn over in the incubation mixture with chromatin, since it is entirely and specifically removed from RNA by phosphodiesterase-free alkaline phosphatase (from calf intestine; Boehringer) (data not shown).

DISCUSSION

We report here that RNA synthesized *in vitro* on chromatin or DNA is partially resistant to digestion by a mixture of RNases A and T1 at high ionic strength. We conclude that this resistant material represents dsRNA regions on the following basis: (*i*) inability of DNase to increase the sensitivity of these sequences to RNase digestion; (*ii*) total susceptibility to RNase III, specific for dsRNA, and insensitivity to RNase H, specific for RNA·DNA hybrids; (*iii*) relative stability of these sequences towards thermal denaturation.

These double-stranded structures seem to be formed by intramolecular base pairing (in "hairpin loop"-like sequences), since considerably more dsRNA is accessible to RNase in high salt at 100° when RNase is present during melting than when the enzyme is added afterwards; this suggests that spontaneous renaturation of RNA takes place after melting, even when the sample is rapidly cooled in ice. The rapidity of reappearance of resistance to RNase, when shifting either from high to low temperature or from low to high salt buffer, is best explained by the "hairpin" hypothesis.

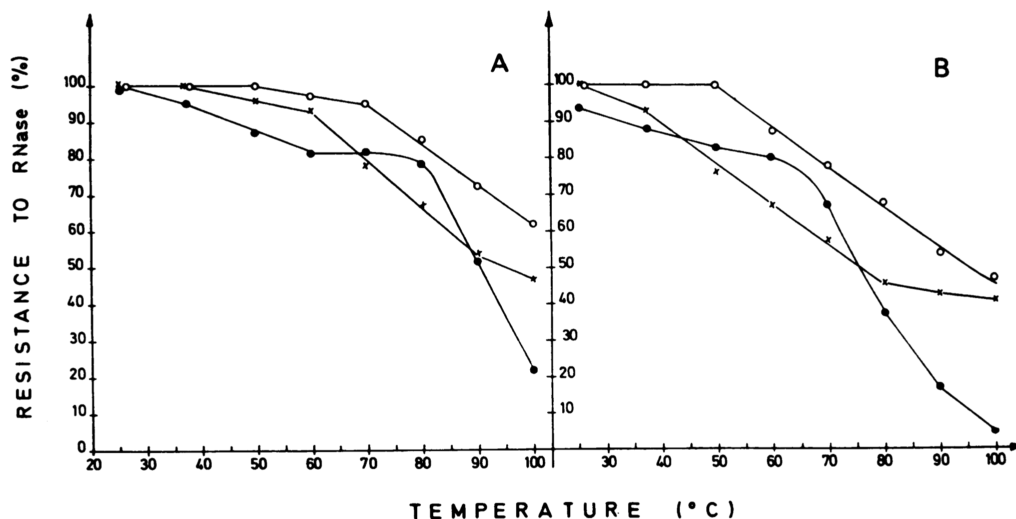

FIG. 5. Thermal denaturation of dsRNA from chromatin transcript. (A) RNA was synthesized on chromatin by rat liver RNA polymerase B and purified as described in *Materials and Methods.* RNA (approximately 1 ng/ml) was heated for 5 min at different temperatures in 10 mM EDTA, 5 mM Tris·HCl (pH 7.9) (×), or in 0.3 M NaCl, 10 mM EDTA, 5 mM Tris·HCl (pH 7.9) (○), then incubated for 2 hr at 37° in high salt, with 100 μg/ml of RNase A and 50 units/ml of RNase T1. (●): RNA was heated for 5 min in 0.3 M NaCl, 10 mM EDTA, 5 mM Tris·HCl (pH 7.9) in the presence of RNases A + T1, then incubated for 2 hr at 37°. (B) Same experiment, but performed in the presence of 40% (vol/vol) formamide. After heating, samples were diluted 10-fold in 0.3 M NaCl, 10 mM EDTA, 5 mM Tris·HCl (pH 7.9), and incubated for 2 hr at 37° with the RNases. Results are expressed as percentages of maximal resistance to RNase.

Table 2. Action of different RNases on chromatin RNA transcripts

	Acid-precipitable RNA	
	cpm	%
Control	2082	100
+ 100 μg of RNase A	945	45.4
+ 100 μg of RNase A and 60 units of RNase H	923	44.3
+ 100 μg of RNase A and 75 units of RNase III	86	4.1
+ 60 units of RNase H	1936	93.0
+ 75 units of RNase III	1137	54.5

Assays were performed as described in *Materials and Methods*, with RNA synthesized *in vitro* on chromatin by rat liver RNA polymerase B. RNase H and RNase III units of activity were as defined in ref. 21.

The dsRNA sequences synthesized by rat liver RNA polymerase B contain about 1.5 to 2 times more cytidine than uridine, which means that G·C pairs are more abundant than U·A pairs. Despite their high G·C content, it is unlikely that these sequences originate from ribosomal RNAs, since 0.1 μg/ml of α-amanitin, a specific inhibitor of the nucleoplasmic RNA polymerase, suppresses by more than 95% the incorporation of nucleotides directed by a chromatin template. It is, however, possible that ribosomal cistrons are transcribed by RNA polymerase B at low ionic strength. Our results can be compared with those of Jelinek and Darnell (18) and Ryskov *et al.* (22), who found a composition close to 30% cytidine and 20% uridine in double-stranded regions of non-ribosomal heterogeneous nuclear RNA from HeLa or ascites cells.

Upon thermal denaturation, the dsRNAs have a T_m of 95° in 0.3 M NaCl (when RNase is present during melting), which is lower than that expected from its relatively high G+C content (19). However, this discrepancy could be accounted for by the shortness of these sequences, the majority of which are only about 20 to 30 nucleotides long. On the other hand, a small fraction of dsRNA (varying from less than 10–30%) is denatured at 43°. This particular fraction might be of very small molecular size, or composed of imperfect duplexes.

Since dsRNA sequences could be used as recognition signals for regulatory elements or enzymes such as nucleases or RNA polymerase, the location of these structures is of a particular interest. We have approached this question by labeling the 5′-terminal nucleotide with nucleoside [γ-^{32}P]triphosphates. Unfortunately, the use of ATP also resulted in ^{32}P incorporation in non alkali-labile molecules, thus not RNA, which were distributed all along 2.7% polyacrylamide gels. Therefore, we used only [γ-^{32}P]GTP, which was uniquely incorporated into RNA. The incorporated GTP was about 30% resistant to RNase digestion in high salt. Thus at least a fraction of RNA chains could be initiated in double-stranded structures. Moreover, the endogenous RNA polymerase activity of chromatin, which is unable to initiate transcription (15, 23), does not synthesize dsRNA. Furthermore, the percentage of double-stranded sequences is much lower in total hnRNA than in chromatin transcripts, which probably consist principally in RNA chain beginnings. These observations, taken together, suggest that the 5′ end of RNA transcripts could be enriched in double-stranded sequences.

On the other hand, the proportion of dsRNA is 10-fold higher in chromatin transcripts by liver RNA polymerase B than in DNA transcripts, and nearly 2-fold higher than in chromatin transcripts by *E. coli* RNA polymerase. Since initiation of transcription is presumed to be more specific on chromatin than on DNA, and with homologous RNA polymerase than with the bacterial enzyme (8, 12), it is suggested that double-stranded structures could be related to correct initiation by RNA polymerase.

I would like to express special thanks to Dr. J. L. Darlix, who kindly provided me pure RNase III and RNase H. I am indebted to Profs. J. Brachet, R. Kram, F. Hanocq, and C. Thomas for helpful discussions and comments on the manuscript. The excellent technical assistance of Mrs. N. Herremans is also acknowledged. I am an "Aspirant du Fonds National de la Recherche Scientifique" (Belgium).

1. Paul, J. & Gilmour, R. S. (1968) *J. Mol. Biol.* **34**, 305–316.
2. Smith, K. D., Church, R. B. & McCarthy, B. J. (1969) *Biochemistry* **8**, 4271–4277.
3. Spelsberg, T. C. & Hnilica, L. S. (1971) *Biochim. Biophys. Acta* **228**, 212–222.
4. Axel, R., Cedar, H. & Felsenfeld, G. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2029–2032.
5. Astrin, S. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2304–2308.
6. Gilmour, R. S. & Paul, J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3440–3442.
7. Shih, T. S., Khoury, G. & Martin, M. A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3506–3510.
8. Steggles, A. W., Wilson, G. N., Kantor, J. A., Picciano, D. J., Falvey, A. K. & Anderson, W. F. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 1219–1223.
9. Jacquet, M., Groner, Y., Monroy, G. & Hurwitz, J. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3045–3049.
10. Keshgegian, A. A., Garibian, G. S. & Furth, J. J. (1973) *Biochemistry* **12**, 4337–4342.
11. Meilhac, M. & Chambon, P. (1973) *Eur. J. Biochem.* **35**, 454–463.
12. Butterworth, P. H. W., Cox, R. F. & Chesterton, C. J. (1971) *Eur. J. Biochem.* **23**, 229–241.
13. Davidson, E. H. & Britten, R. J. (1973) *Quart. Rev. Biol.* **48**, 565–613.
14. Pays, E. & Ronsse, A. (1975) *Biochem. Biophys. Res. Commun.* **62**, 862–867.
15. Pays, E. & Ronsse, A. (1973) *Eur. J. Biochem.* **40**, 119–131.
16. Groner, Y., Monroy, G., Jacquet, M. & Hurwitz, J. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 194–199.
17. Loening, U. E. (1967) *Biochem. J.* **102**, 251–257.
18. Jelinek, W. & Darnell, J. E. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2537–2541.
19. Van Griensven, L. S. L. D., Van Kammen, A. & Rezelman, G. (1973) *J. Gen. Virol.* **18**, 359–367.
20. Jelinek, W., Molloy, G., Fernandez-Munoz, R., Salditt, M. & Darnell, J. E. (1974) *J. Mol. Biol.* **82**, 361–370.
21. Darlix, J. L. (1975) *Eur. J. Biochem.* **51**, 369–376.
22. Ryskov, A. P., Yenikolopov, G. N. & Limborska, S. A. (1974) *FEBS Lett.* **47**, 98–102.
23. Cox, R. F. (1973) *Eur. J. Biochem.* **39**, 49–61.