Transcription of DNA-histone complexes by yeast RNA polymerase B

L.K. Karagyozev, M.A. Valkanov and A.A. Hadjiolov

Department of Molecular Genetics, Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Received 5 April 1978

ABSTRACT

Transcription of denatured DNA complexed with histones (total, H1 or H2A/H2B/H3/H4) by yeast RNA polymerase B is investigated. Binding of histones to DNA restricts its template activity by decreasing the formation of active, heparin-resistant, RNA polymerase initiation complexes. The elongation of pre-initiated RNA on denatured DNA, complexed with histones, is possible, although resulting in somewhat shorter RNA chains. It is suggested that RNA polymerase B can elongate on a DNA strand covered with histones.

INTRODUCTION

It is established that histones strongly inhibit the template activity of native DNA transcribed in vitro (1). Experiments with native or modified chromatin show also that it is a poorer template than DNA (1,2). The mechanism of restriction in chromatin and DNA-histone complexes is not well understood. Evidence is obtained that these templates contain fewer RNA polymerase initiation sites (3-5). It is reported also that elongation on restricted templates is possible, although at a decreased rate (6-8). However, it is not yet clarified whether the transcribed DNA chain is associated with histones during progression of RNA polymerase. In this work we studied the transcription of DNA-histone complexes by yeast RNA polymerase B. The experiments were carried out under conditions permitting discrimination between the initiation and elongation steps of RNA synthesis. Our results show that elongation of pre-initiated RNA chains is possible on a denatured DNA template complexed with histones.

MATERIALS AND METHODS

Isolation of yeast RNA polymerase B - RNA polymerases are extracted from the osmotic-sensitive S.cerevisiae strain VY 1160 cultivated in the standard YPD 5 medium containing 10 % sorbitol (9). The late log phase cells are lysed in distilled water and sonicated 4-fold for 20 sec. with a MSE ultrasonic disintegrator in a medium containing 0.24 M $(\text{NH}_4)_2\text{SO}_4$, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 10 mM MgCl_2 , 10 mM β -mercaptoethanol and 10 % glycerol at 4°C. All subsequent manipulations are carried out at 4°C with pre-cooled solutions. Cell debris are sedimented for 10 min. at 2000xg. Polyethyleneglycol-6000 dissolved in 0.24 M $(\text{NH}_4)_2\text{SO}_4$ and TEM buffer (20 mM Tris-HCl, pH 7.9; 0.5 mM EDTA; 10 mM β -mercaptoethanol) is added to the supernatant to a final concentration of 10 %. The mixture is centrifuged for 90 min. at 48000xg in a Beckman JA-21B centrifuge. The clear supernatant is mixed with a saturated solution of $(\text{NH}_4)_2\text{SO}_4$ to a final concentration of 2.4 M. After 30 min. the protein precipitate is collected by centrifugation for 10 min. at 25000xg and dissolved in TEM buffer containing 25 % glycerol. The fractionation of RNA polymerases A,B and C is achieved by DEAE-Sephadex A-25 column chromatography according to Adman et al. (10). Elution is carried out with an $(\text{NH}_4)_2\text{SO}_4$ gradient in TEM buffer containing 25 % glycerol. The peak of RNA polymerase B is concentrated on a small DEAE-Sephadex A-25 column (10). The preparations of RNA polymerase B display a marked preference for denatured DNA as template, they are fully inhibited by α -amanitin (30 $\mu\text{g}/\text{ml}$) and show optimal activity at 75 mM $(\text{NH}_4)_2\text{SO}_4$ (10-12). The RNA polymerase B preparations are stored in TEM buffer containing 25 % glycerol and 0.3 M $(\text{NH}_4)_2\text{SO}_4$ at -20°C.

Assay of RNA polymerase activity - The enzyme activity is determined essentially as described earlier (13). The standard incubation mixture contains in 60 μl : 50 mM Tris-HCl (pH 7.9); 2 mM MnCl_2 ; 0.24 mM each of ATP, GTP and CTP; 0.016 mM ^3H -UTP (2 Ci/mmol); 80 $\mu\text{g}/\text{ml}$ heat-denatured calf thymus DNA and 20 μl enzyme in TEM buffer. After incubation for 20 min. at 30°C 50 μl samples are pipeted on to 2.4 cm. Whatman 1 filter-paper discs (pre-soaked with 50 μl 0.1 M EDTA and dried). After processing

as described (13) the radioactivity of the acid-insoluble product is measured in a Packard Tri-Carb model 3320 liquid scintillation spectrometer. The reaction is linear for at least 20 min. One unit of RNA polymerase is equivalent to the incorporation of one nmole ^3H -UTP into acid-insoluble material for 20 min. at 30°C .

Discrimination between initiation and elongation of RNA chains - The formation of the initiation complex is based on the procedure of Hyman and Davidson (14). The enzyme is incubated with heat-denatured T_7 phage or calf thymus DNA in the presence of 50 mM Tris-HCl (pH 7.9); 2 mM MnCl_2 ; 0.3 mM each of ATP and GTP; 0.06 mM ^3H -UTP; 40 mM $(\text{NH}_4)_2\text{SO}_4$; 10 % glycerol and 5 mM β -mercaptoethanol. Incubation for 15 min. at 30°C is sufficient for the formation of all initiation complexes (see Results). Elongation is started by the addition of CTP to 0.3 mM and carried out at 30°C for the indicated time. In most experiments the total volume is 70 μl . Heparin or histones are added before starting elongation.

Preparation of histones and DNA-histone complexes - Rat liver chromatin is isolated according to Tsanev and Russev (15). Histones and histone fractions are prepared by the method of Oliver et al.(16). The purity of fractionated histones: H1 and H2A/H2B/H3/H4 is determined by acrylamide gel electrophoresis (17). Concentration of the histone stock solutions is determined by spectrophotometry (18) and by the method of Lowry et al.(19) with bovine serum albumin as standard.

The preparations of DNA are denatured by heating for 10 min. at 100°C in glass-distilled water and cooled in an ice bath. DNA-histone complexes are prepared by rapid mixing of heat-denatured DNA and histone solutions in glass-distilled water at room temperature. The template activity of DNA-histone complexes (stored at 4°C) is determined 12 h after preparation. The same procedure is followed in the preparation of DNA-poly-L-lysine complexes.

Acrylamide-agarose gel electrophoresis - The RNA polymerase reaction mixture is treated for 60 min. at 25°C with 100 $\mu\text{g}/\text{ml}$ deoxyribonuclease I in the presence of 5 mM MnCl_2 and 75 $\mu\text{g}/\text{ml}$ of 28 S rRNA from Ehrlich ascites tumour cells. Sodium dodecylsulphate (to 0.5 %) and formamide (to 60 %) are added and

the mixture is heated for 3 min. at 70°C. Electrophoresis of the RNA synthesized in vitro is carried out in 3 % acrylamide-agarose gels (20) in buffer E (21) with rat liver 18 S, 5 S and 4 S RNA as standards. The gels are washed for 48 h in 10 % acetic acid, cut in 4 mm slices and their radioactivity determined as described earlier (22).

Materials - Analytical grade reagents are used throughout. The E.coli T₇ phage is prepared according to Iamamoto et al.(23) and its DNA isolated and purified by the phenol-chloroform method (24). Calf thymus DNA ("highly polymerized"), ATP, GTP, CTP and UTP are from Sigma Chem.Co, St. Louis, U.S.A. ³H-UTP is a product of The Radiochemical Centre, Amersham, U.K.; heparin of Schwarz/Mann, Orangeburg, N.Y., U.S.A. and polyethylene glycol-6000 of Merck, Darmstadt, B.R.D. Deoxyribonuclease I (electrophoretically pure) is from Worthington Biochem. Corp., Freehold, N.J., U.S.A.; α -amanitin from Calbiochem, U.S.A. and poly-L-lysine from Koch-Light, U.K.

RESULTS

Inhibition of the template activity of denatured DNA by histones

Complexing of denatured DNA with histones or poly-L-lysine inhibits markedly its template activity (Fig.1). A similar inhibition of template activity of double-stranded DNA by histones or poly-L-lysine was reported (i.e.25,26). It is known (27) that these basic polypeptides are tightly bound to single-stranded DNA under the ionic conditions of the RNA polymerase reaction used in our experiments. Therefore, binding of histones and poly-L-lysine to denatured DNA restricts its template activity.

The observed restriction may be due to the following major factors: (a) RNA polymerase cannot bind to DNA initiation sites complexed with histones; (b) the enzyme binds to the DNA-histone template, but cannot form active initiation complexes and (c) the enzyme can form initiation complexes, but elongation is impossible. We attempted to discriminate the contribution of the above factors in the observed restriction of DNA template activity by histones.

Elongation of RNA chains without reinitiation

Incubation of RNA polymerase with a template in the presence

Figure 1. Inhibition of the template activity of denatured DNA by histones and poly-L-lysine. The complexes between heat-denatured calf thymus DNA and protein are added to the standard mixture for RNA polymerase containing 80 mM $(\text{NH}_4)_2\text{SO}_4$ (Methods). The concentration of DNA is 17 $\mu\text{g/ml}$. A - total histone; B - histone H1; C - histones H2A/H2B/H3/H4; D - poly-L-lysine.

of GTP, ATP and UTP results in the formation of short oligonucleotide chains at each initiation site (14). Addition of CTP permits elongation of the initiated chains. Heparin inactivates the free enzyme, but elongation of pre-initiated chains is possible in the case of RNA polymerase B transcribing chromatin or double-stranded DNA (28,29). We studied the effect of heparin on the transcription of denatured DNA by yeast RNA polymerase B. The results (Fig.2A) show that in this case heparin inactivates the free enzyme too, but allows elongation of pre-initiated RNA chains. We used this property of heparin to study the kinetics of initiation. The results (Fig.2B) demonstrate that preincubation for 15 min. is sufficient for the formation of all possible heparin-resistant initiation complexes. Thus, in the presence of heparin yeast RNA polymerase bound to denatured DNA completes only one transcription cycle and elongates a single pre-initiated RNA chain.

Elongation of RNA chains on complexes of denatured DNA with histones

The possibility of elongation of RNA chains on DNA-histone templates is studied by the addition of histones to pre-formed RNA polymerase initiation complexes. Here, the histone/DNA ratio

Figure 2. A/ Inactivation of free and initiated RNA polymerase B by heparin. (●—●) - The enzyme is mixed with heparin and assayed in the standard incubation mixture (see Methods). Incubation time - 10 min. (▲—▲) - RNA polymerase initiation complexes are formed as described in Methods and mixed with heparin. Incubation time after addition of CTP - 10 min.

B/ Kinetics of initiation. RNA polymerase (1.4 units) is incubated with heat-denatured T₇ DNA (2.4 μg) as described in Methods in a total volume of 300 μl . At various periods of time aliquots (50 μl) are taken, mixed with heparin (18 $\mu\text{g/ml}$) and the reaction started by the addition of CTP. Incubation - 15 min.

is markedly higher than that needed for complete block of transcription (see Fig.1).(Addition of histones to DNA in a mass ratio higher than 3 results in the complete inhibition of template activity.) The results of one such experiment are given in Table 1.

The results demonstrate that elongation of pre-initiated RNA chains on a single-stranded DNA complexed with histones is possible. The amount of product is similar to that made in the presence of heparin. In both cases reinitiation is abolished and this indicates that an almost equal number of RNA polymerase molecules elongate and produce a product of similar size. The block of elongation caused by poly-L-lysine suggests that in this case steric hindrance is imposed on RNA polymerase propagation.

The time course of elongation of pre-initiated RNA chains in the presence of heparin or histone H1 is shown in Fig.3. It is clearly seen that elongation is possible in the presence of either heparin or histone H1. In both cases the reaction reaches

Table 1. Elongation of pre-formed RNA chains on DNA-histone complexes

Addition to pre-initiated enzyme mix	Protein/DNA ratio (w/w)	pmoles ^3H -UMP incorporated per sample	
		5 min.	15 min.
Heparin(18 $\mu\text{g/ml}$)	--	25	56
Total histones	5.7	21	42
H2A/H2B/H3/H4	6.4	20	39
H1	7.0	22	44
Poly-L-lysine	4.5	0.02	0.02

RNA polymerase B (1.6 units) is incubated with 9 μg denatured T_7 DNA in the presence of ATP, GTP and ^3H -UTP for 15 min. at 30°C (see Methods). To aliquots of the mixture the indicated additions are made and incubation continues for another 30 min. at 4°C . Elongation is started by addition of CTP and incorporation of ^3H -UMP into acid-insoluble product is measured. The results are corrected for zero-time incorporation of ^3H -UMP.

plateau levels in about 15 min.

The RNA product made at the end of elongation (Fig.4) is heterogeneous in size with an average length of about 400-500 nucleotides. However, the relative amount of shorter RNA chains is higher when elongation proceeds on DNA-histone H1 complexes.

Figure 3. Time course of elongation of RNA chains in the presence of heparin (●) or histone H1 (○). RNA polymerase (1.5 units) is mixed with heat-denatured T_7 DNA (7 μg) and the components needed for the formation of initiation complexes (see Methods). After 15 min. incubation the mixture is divided in two parts. One is mixed with heparin (18 $\mu\text{g/ml}$) and the other with histone H1 (21 μg). After 30 min. at 4°C elongation is started at zero time by the addition of CTP. Incorporation of ^3H -UMP is measured in aliquots taken at different time intervals and expressed as pmoles ^3H -UMP incorporated per tube.

Figure 4. Gel electrophoresis of the product obtained by elongation of pre-initiated RNA chains in the presence of histone H1 (A) or heparin (B). The experimental conditions for the RNA polymerase reaction are as in Fig.3. Elongation is started by the addition of CTP and carried out for 15 min. Acrylamide-agarose gel electrophoresis analysis of the product is as described in Methods.

This may be correlated with the observation that the RNA product formed on this template is approximately 25 % less (see Fig.3) and suggests that earlier termination of RNA chains may occur on DNA complexed with histones.

Titration of yeast RNA polymerase B with template

To study further the observed restriction of the template activity of heat-denatured DNA by histones, we performed experiments to estimate the availability of different templates (6). Titration of a constant amount of yeast RNA polymerase B is carried out with increasing amounts of naked denatured DNA or denatured DNA complexed with histone H1 (histone/DNA ratios (w/w) of 0.85 and 1.66). The enzyme-template mixtures are incubated with the three nucleoside-5'-triphosphates (see Methods) and the number of correct initiation complexes is evaluated by the amount of product obtained upon elongation in the presence of heparin. As known, heparin blocks reinitiation and removes histones from DNA (31,32). Therefore, the RNA product synthesized under these conditions will depend only on the number of pre-initiated RNA chains. The results (Fig.5)

Figure 5. Titration of RNA polymerase with DNA and DNA-histone H1 complexes. Increasing amounts of heat-denatured T₇ DNA (●—●) or the same DNA complexed with histone H1 in a histone/DNA ratio (w/w) of 0.85 (▲—▲) or 1.66 (○—○) are added to RNA polymerase (0.25 units). After incubation for 15 min. under standard conditions (see text) heparin (40 μg/ml) and CTP are added. Incorporation of ³H-UMP is assayed after 15 min. elongation of pre-initiated RNA chains.

demonstrate that the amount of active initiation sites decreases upon complexing of heat-denatured DNA with histones. The lower plateau levels observed with DNA-histone templates show also that the enzyme binds to the template, but forms heparin-sensitive complexes. A similar formation of inactive complexes of RNA polymerase with chromatin and DNA-histones has been observed earlier (6,26,32,33).

DISCUSSION

It is known that transcription requires local unwinding of the double-stranded DNA template (34,35). With chromatin templates it is not yet clear whether histones remain bound to the transcribed DNA chain. This possibility is put forward in a model of chromatin transcription proposed by Weintraub et al. (36). Our results provide experimental evidence that elongation of pre-initiated RNA chains is possible on a single-stranded DNA covered with histones. The restriction of single-stranded DNA templates by histones, as well as analysis of the amount and size of the product, make unlikely the possibility that long

stretches of naked DNA remain open for transcription. However, our experiments do not solve the problem whether or not histones are removed from the template by the enzyme as transcription proceeds. We consider more likely that histones bind to the DNA backbone leaving the DNA bases essentially free for the interactions involved in RNA elongation. Thus, transcription on a DNA strand covered with histones would resemble the case where synthesis of DNA takes place on a single-stranded DNA complexed with a tightly bound protein (37).

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