TRANSCRIPTION OF POLYOMA VIRUS DNA AFTER INTERACTION WITH NUCLEAR PROTEINS IN VITRO

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Summary : RNA polymerase B, histones and non-histone chromosomal proteins (NHP) extracted from calf thymus nuclei were used for transcription experiments with superhelical form I and linear Eco R1 cleaved Polyoma virus DNA. NHP added to DNA with a NHP/DNA weight ratio of 0.5/1 partially inhibited transcription of superhelical DNA but did not affect transcription of linear DNA. The preferential inhibition of form I DNA transcription could be explained by a higher affinity of NHP for this form of DNA: NHP added in excess increased the sedimentation coefficient of superhelical but not of linear DNA. Histones completely inhibited transcription of superhelical DNA, while they only partially inhibited transcription of linear DNA at a histones/DNA ratio of 0.6/1. At the same histones/DNA ratio a modification of the sedimentation properties of linear, but not of superhelical DNA was observed.

In recent years, intensive studies have been devoted to chromatin structure and chromatin transcription (1, 2, 3, 4, 5): one of the aims of this kind of studies is to gain some insights into the problem of how gene expression in eukaryotic cells is regulated at the molecular level. Proteins normally found associated with DNA in chromatin most likely interfere with transcription and are therefore good candidates as possible regulatory elements. However, no clear evidence as to how nuclear proteins might perform a regulatory action has been obtained. Interpretation of in vitro transcription of cellular chromatin in terms of interactions of nuclear proteins with DNA is made difficult by the complexity and size heterogeneity of the template used. Small, well characterized viral DNAs are also associated with nuclear proteins in the nuclei of infected cells in a structure identical to that of chromatin (6, 7). Thus in vitro interaction of these small DNAs with nuclear proteins is a

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relatively simple model system for the study of protein-DNA interaction in the nucleus and its effect on transcription. In the present report the effects of histones and NHP on transcription of an animal tumor virus DNA are shown to differ and to change if linear instead of superhelical DNA is utilized as template.

Materials and Methods

Polyoma DNA was purified essentially as described by Cuzin et al. (8). To obtain linear Py DNA, superhelical DNA was incubated for 2 hrs at 37° with Eco R1 (the kind gift of Dr. M. Yaniv) in 800 µl of 10 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$. ^3H thymidine labeled DNA had a specific radioactivity of 225000 cpm/µg.

RNA polymerase B was purified by a modification of the procedure of Kedinger et al. (9). Details of the purification procedure are to be published elsewhere. The enzyme had a specific activity of about 20 units/mg protein, and it contained no appreciable DNase activity.

Nuclear proteins were prepared essentially as described by Van den Broek et al. (10). When analysed by SDS gels (11), 60 µg of purified NHP gave rise to at least 23 bands. Purified histones were identified by spectral analysis (12) and by polyacrylamide gel electrophoresis in 6M urea (13): all five classes were present. No cross-contamination of the two classes of nuclear proteins was observed. H1 depleted histones were obtained by chromatin extraction with 10 mM Tris-HCl pH 7.9, 50 mM $NaHSO_3$ and 0.5 M NaCl to selectively dissociate H1, 0.2M H_2SO_4 extraction and ethanol precipitation. Residual H1 was practically eliminated by ammonium sulfate precipitation at 70% saturation (H1 did not precipitate).

Transcription assay - The reaction mixture for RNA synthesis contained: 40 mM Tris-HCl pH 7.9, 0.4 mM EDTA, 4 mM DTT, 3 mM $MgCl_2$, 40 mM ammonium sulfate, 6% glycerol, 0.5 mM each ATP, GTP and CTP, 0.1 mM ^3H-UTP (500 µCi/µM). Reaction mixtures (150 µl) containing the specified amounts of DNA and nuclear proteins were preincubated for 5 minutes at 37°, and the reaction was started with the addition of 2 µl of the enzyme (0.2 units; at this enzyme/DNA ratio the reaction was in the linear portion of a DNA dose-response curve for all DNAs used). At the indicated times, 20 µl aliquots were taken and precipitated by addition of ice cold 5% trichloroacetic acid, 1% Na pyrofosphate. After 20 minutes, the precipitates were collected on Whatman GF/C filters, washed with about 40 ml of TCA-pyrophosphate, 20 ml of cold 10 mM HCl and 5 ml of ethanol, dried and counted in a Beckman scintillation counter.

Sedimentation studies - The indicated amounts of DNA and nuclear proteins were mixed in a final volume of 100 µl containing 10 mM Tris-HCl pH 7.9, 10 mM NaCl, and left at 22° for 10 minutes. They were then layered on top of 5-20% sucrose gradients in 10 mM Tris-HCl pH 7.9, 10 mM NaCl, 0.2 mM EDTA. Gradients were centrifuged as specified in the figure legends. Fractions were collected directly on GF/C filters that were batch-washed in TCA-pyrophosphate, rinsed twice with ethanol, dried and counted. Approximate sedimentation coefficient calculations were based on

assumption of 21S for superhelical and 16S for nick-relaxed Py DNA and of constant sedimentation velocities.

Results and Discussion

To study the effect of nuclear proteins on transcription, Py superhelical or linear DNA was preincubated with NHP or histones, then eukaryotic RNA polymerase B was added and kinetics of incorporation were followed for 30 minutes. In the presence of NHP at a protein to DNA weight ratio of 0.5 transcription of Py form I DNA was inhibited by 30 per cent on average (Fig. 1A), while the same amount of NHP had no effect of linear DNA transcription (Fig. 1B). When NHP were added to Py DNA at a 1:1 ratio, inhibition of transcription was 50% (result not shown). Total histones added at a proteins/DNA ratio of 0.6 inhibited transcription of superhelical DNA completely (Fig. 1A) and of linear DNA 45% (Fig. 1B). To check the role of histone H1 in transcription inhibition, H1-depleted histones were prepared and added to form I Py DNA in similar transcription experiments. At a proteins/DNA ratio of 1, 82% inhibition was still observed (data not shown), suggesting that H1 histone was neither the only nor the main responsible for the observed histone effect on transcription. When NHP where preincubated with superhelical Py DNA before addition of H1-depleted histones, the transcription curve was almost identical to that obtained with

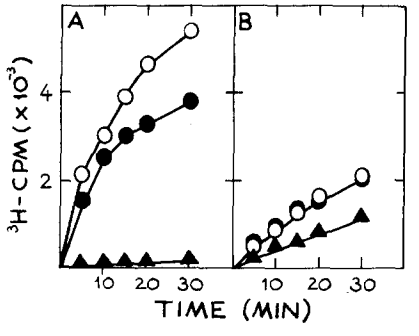

Fig. 1 - Effect of His and NHP on transcription of superhelical and linear Py DNA. Reaction mixtures contained 2 µg of DNA and, when present, 1 µg of NHP or 1.2 µg of His in a final volume of 150 µl (for details see Materials and Methods). (A) superhelical Py DNA; (B) Linear Py DNA. Symbols : O—O no nuclear proteins added; ●—● NHP added; ▲—▲ His added.

the four histones alone (data not shown). The inhibitions by NHP and H1-depleted histones were thus not additive in these experiments, but competitive, and the interaction with the four histones appeared to be the dominant one.

Taken together these results suggest that at the proteins/DNA weight ratios used in these experiments, interaction of NHP and His with superhelical DNA was either greatly favoured or just much more effective in inhibiting transcription. The different effect of nuclear proteins on transcription of the two forms of DNA argues in any case against a direct effect on RNA polymerase. The interaction of histones and NHP with linear and superhelical DNA was thus further investigated by sedimentation analysis. Highly radioactive DNA was mixed with different amounts of nuclear proteins in 10 mM Tris pH 7.9, 10 mM NaCl and left at room temperature for 10 minutes before centrifuging through a linear 5-20% sucrose gradient in the same buffer. Because of its high specific radioactivity, the DNA consisted of a mixture of superhelical and circular relaxed molecules, which were separated on the gradient. (Unlabeled Py DNA used as a template in transcription experiments contained less than 10 per cent nicked circles). Information on the binding to both DNA form I and II was thus obtained from the same sample.

When NHP were mixed with DNA in the same ratios used in transcription experiments no variation in the sedimentation pattern was observed (data not shown). This showed that the effect of NHP on transcription was not due to a gross modification of the state of the template. Assuming an average molecular weight of 30,000 daltons for NHP, a NHP/DNA weight ratio of 0.5 would correspond to about 50 protein molecules added (we have no measure of the binding efficiency in our assay conditions) per molecule of Py DNA. At a NHP/DNA ratio of 7.5 a slight displacement of the form I peak towards the bottom of the tube was observed, whereas the position of the nicked circle was the same as in the control (Fig. 2B). At a NHP/DNA ratio of about 40, both form I and form II were clearly displaced (Fig. 2C): the superhelix showed a more heterodisperse peak around 25S, whereas form II sedimented at about 18S. No radioactivity was found at the bottom of the tube, so that formation of faster sedimenting aggregates or complexes

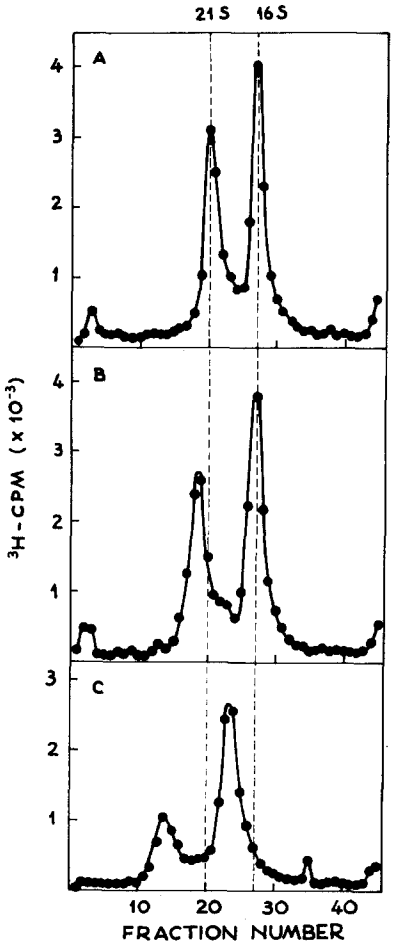

Fig. 2 - Interaction of NHP with superhelical and circular relaxed Py DNA. ^{3}H Py DNA (0.13 µg) and the amounts of NHP indicated below were mixed as described in Materials and Methods and then centrifuged at 48,000 rpm for 135 minutes in a Spinco SW 50 L rotor at 5°. Fractions were collected and processed as described in Materials and Methods.
(A) No NHP added; (B) 1 ug NHP added; (C) 5 ug NHP added.

could be excluded. The fact that binding of NHP modified more extensively the hydrodynamic properties of superhelical Py DNA than those of the relaxed form suggests that binding to form I DNA might be preferential with respect to form II. When the same experiment was performed with linear Py DNA, no modification appeared in the homogeneous sedimentation pattern, even when a NHP/DNA ratio of 60 was used (Fig. 3A and B). NHP

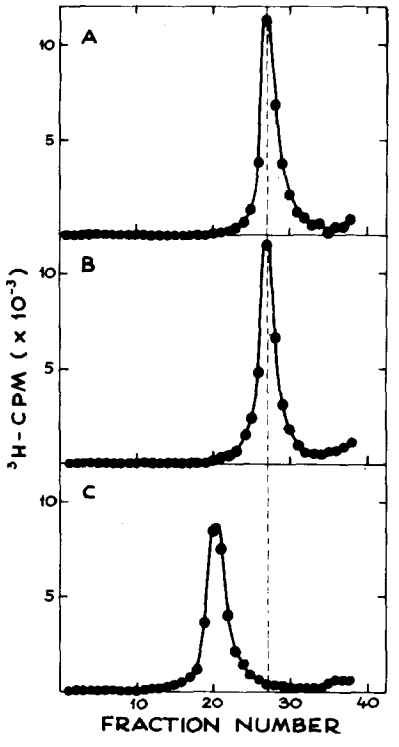

Fig. 3 - Interaction of NHP and His with linear Py DNA. Linear ^{3}H Py DNA (0.12 µg) was mixed with NHP or His as described in Materials and Methods and then centrifuged at 55,000 rpm for 110 minutes in a Spinco SW 56 rotor at 4°. Fractions were collected and processed as described in Materials and Methods. (A) no nuclear proteins added; (B) 7.5 µg NHP added; (C) 0.075 µg His added.

binding to superhelical and linear Py DNA, as measured by the increase in DNA sedimentation velocity, could therefore be correlated to the effect of NHP on transcription, in that NHP bound preferentially to the DNA species (the superhelix) whose transcription they affected most.

When histones were mixed with form I and II Py DNA at a histones/DNA ratio of 0.6 (at which ratio transcription of the superhelix was completely inhibited as shown in Fig. 1A), no modification of the DNA sedimentation pattern was observed (data not shown). As the binding conditions were slightly different from those used for transcription, we repeated the experiment in the same ionic medium used in the transcription assay (triphosphates were not added), and centrifuged through a 5-20% sucrose gradient

in the same medium. Again, histones caused no change in the sedimentation pattern of Py DNA (data not shown), showing that the observed transcription inhibition was not due to DNA aggregation and precipitation. At higher histones/DNA ratios large aggregates were formed, which sedimented at the bottom of the tube even after short centrifugation times; precipitation occured also when H1-depleted histones were used alone, or added on to a DNA-NHP soluble mixture. When histones were mixed with linear Py DNA at a histones/DNA ratio of 0.6, an increase in sedimentation velocity was observed (Fig. 3C). By comparing the results of histone experiments with linear and circular DNA, several conclusions could be drawn. First, histones bind preferentially (at last as shown by sedimentation analysis) to linear Py DNA with respect to the superhelical and circular nicked forms. Second, the complete inhibition of transcription of Py DNA by histones is probably not the result of DNA aggregation but of histones-DNA interaction in solution. Third, interaction with histones, even when not detected by sedimentation, seems to be much more effective in blocking RNA polymerase action on superhelical than on linear DNA.

In conclusion our results show that the two classes of nuclear proteins, histones and NHP, have different effects on transcription of Py DNA, depending on the physical state of the DNA molecule.

Recently, several laboratories have provided physical, electron microscopic and biochemical evidence that both cellular and viral DNAs are complexed with histones in discrete repeating units called "nucleosomes" (6, 7, 14, 15, 16, 17, 18). Most likely we are not dealing here with this kind of structures, since our experimental conditions would not allow nucleosome reconstruction. DNA arranged in nucleosomes seems to be inactive in transcription (Chambon, personal communication): this suggests that nucleosomes must be disrupted in order for transcription to occur. In this respect, studies of direct interaction of nuclear proteins with DNA are useful to understand the nature of the cellular or viral transcription template.

Acknowledgement

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