CHARACTERISTICS OF RNA SYNTHESIS ON MUSTINE-DAMAGED TEMPLATES

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Bifunctional alkylating compounds, by their action on the genetic apparatus of the cell, can not only injure DNA, but can also disturb the native character of DNA–protein bonds in the composition of chromatin. Some workers consider that the role of one or other factor is predominant in producing a change in the transcription activity of the genome [4, 7, 8].

The object of this investigation was to differentiate the contribution of injured DNA to the change in template activity of chromatin and to establish the cause of the reduction in RNA-synthesizing capacity of DNA and chromatin [3] after the action of the bifunctional alkylating agent mustine on them.

EXPERIMENTAL METHOD

Di-(2-chloroethyl)-methylamine (mustine) was used as the hydrochloride. Preparations of chromatin were obtained from rat liver nuclei by disintegrating them in 0.024 M EDTA, 0.075 M NaCl, pH 8.0, followed by repeated washing in Tris-HCl buffer, pH 8.0, of decreasing concentrations. DNA was isolated from chromatin by centrifugation for 36 h at 105,000g after dissolving in 2 M NaCl, 5 M urea. Treatment of the DNA (1 mg/ml) or chromatin preparations with mustine (10^{-4} M), RNA synthesis *in vitro* with RNA-polymerase from *E. coli* and determination of the concentrations of DNA and protein were carried out as described previously [3], and sedimentation analysis of RNA by the method in [1]. Reconstruction of DNP from total chromatin protein and DNA treated (or not treated) with mustine was carried out by dialysis of a mixture of DNA and protein in 0.4 M guanidine hydrochloride in 6 M urea against decreasing concentrations of NaCl in 5 M urea and 50 mM Na acetate, pH 6.0, by the following scheme; for 1 h in 1.5, 0.9, 0.6, 0.4, 0.3, 0.2, and 0.1 M, then overnight against urea in Na-acetate buffer, followed by 4 h against 10 mM Tris-HCl buffer, pH 8.0. Phenylmethylsulfanyl fluoride was present in all solutions. Electrophoresis of the chromatin proteins was carried out by Laemmli's method [6]. The sedimentation calculation were based on Osterman's nomograms [2] and the number of RNA molecules was determined by the method of Cedar and Felsenfeld [5]. The mean sedimentation constant was calculated by the equation

$$\overline{S}_{20,\omega} = \frac{\sum_i N_i S_{i,20,\omega}}{\sum_i N_i},$$

and the mean molecular weight by the equation

$$\overline{M} = 1550 \frac{\sum_i N_i S_{i,20,\omega}^{2,1}}{\sum_i N_i},$$

where N_i is the radioactivity of the i-th fraction of the sucrose gradient and $S_{i,20,\omega}$ the sedimentation constant of RNA for this fraction.

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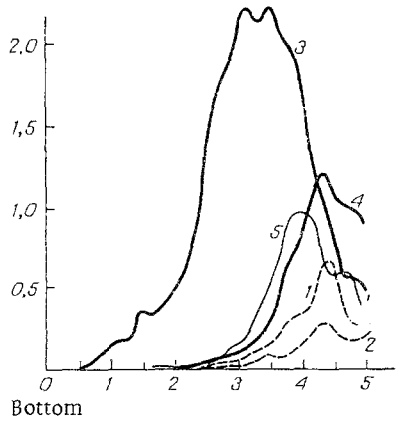

Fig. 1. Sedimentation profiles of RNA synthesized on chromatin and on DNA isolated from it. 1) Control chromatin; 2) chromatin treated with mustine; 3) control DNA obtained by salt deproteinization of control chromatin; 4) DNA obtained by salt deproteinization of chromatin treated with mustine; 5) the same DNA, but additionally treated with pronase. Abscissa, volume of gradient (in cm^3); ordinate, radioactivity (in $cpm \cdot 10^{-3}$)

TABLE 1. Characteristics of RNA Synthesized on Mustine-Injured Chromatin and DNA Isolated from It

Template for RNA synthesis	Relative mol. wt.	Relative number of DNA molecules
	% of corresponding control	
Control chromatin	100	100
Chromatin treated with mustine	79±23	54±10
DNA from control chromatin	100	100
DNA from chromatin treated with mustine	20±1	121±17
The same DNA, but treated additionally with pronase	41±9	77±7

EXPERIMENTAL RESULTS

The results of sedimentation analysis of RNA in a 5-20% sucrose density gradient are given in Fig. 1. The area below each curve reflects the total quantity of RNA synthesized. The quantity of RNA synthesized was significantly reduced on templates of DNA and chromatin after treatment with mustine. RNA synthesized on DNA isolated from chromatin not treated with mustine was characterized by a mean sedimentation constant of 13.4S (Fig. 1, 3). An RNA with a mean sedimentation constant of 5.4S was synthesized on DNA isolated from chromatin treated with mustine (Fig. 1, 4).

The authors have shown that DNA–protein "cross-linkages" sharply reduce the template activity of chromatin [3]. It might be supposed from this that the decrease in the mean sedimentation constant of RNA was due to the presence of a protein not dissociating from DNA in 2 M NaCl, 5 M urea. To test this hypothesis, DNA, already proteinized in a medium of high ionic strength, was subjected to treatment with pronase. Although the sedimentation constant was thereby increased (7.3S, Fig. 1, 5), it did not reach the control level. The decrease in the sedimentation constant of RNA can therefore be attributed only partially to protein attached by mustine to the DNA template. Alkylation of the DNA itself contained in the chromatin may evidently play an essential role in reducing the length of the RNA chains. Knowing the distribution of the sedimentation constants of RNA, it is possible to calculate their mean molecular weight also. During synthesis on templates of the control DNA and chromatin preparations the mean molecular weight of the RNA was 400,000 and 110,000, respectively.

The mean molecular weights of RNA synthesized on chromatin treated with mustine and on DNA isolated from it are shown in Table 1 (in percentages of the control). The same table gives the relative number of RNA molecules synthesized on mustine-treated templates. The mean molecular weight of RNA synthesized on DNA and the relative number of RNA molecules synthesized on chromatin were reduced statistically significantly after treatment with mustine.

In the experiments described above chromatin — a complex of DNA with proteins — was treated with mustine. To distinguish the effects on RNA synthesis due only to injuries to DNA in the deoxyribonucleoprotein complex, DNP reconstruction experiments were carried out. Chromatin proteins obtained by dissociation of chromatin in solutions with high concentrations of salts and urea were used. The protein spectra of the DNP (obtained by electrophoresis withsodium dodecylsulfate), reconstructed both from the original DNA and from mustine-treated DNA, were identical with the protein spectra of the chromatin. However, the template activity of DNP obtained by reconstruction from treated DNA was not more than 20-30% of that of the reconstructed control preparations of DNP.

The writers previously demonstrated the important role of DNA—protein "cross-linkages" in the reduction of transcription on chromatin treated with bifunctional alkylating agents [3]. The results of the present investigation are evidence that the action of mustine on DNA in the composition of chromatin can lead to similar dramatic consequences. The changes in transcription activity observed under these circumstances take place because of a decrease in the number of synthesized RNA molecules and a decrease inthe molecular weight of the chains.

The phenomenon of the decrease in RNA-synthesizing power of chromatin after treatment with the bifunctional alkylating agent mustine cannot therefore be ascribed predominantly to any one type of injury to the nuclear genome.

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