Effects of Selective Extraction of Histones on Template Activities of Chromatin by Use of Exogenous DNA and RNA Polymerases

(DNA polymerase/RNA polymerase/isolated thymus nuclei/histones)

ALFRED E. MIRSKY AND BERT SILVERMAN

The Rockefeller University, New York, N.Y. 10021

Contribution by Alfred E. Mirsky, April 26, 1973

ABSTRACT Exogenous DNA and RNA polymerases were used to measure the template activity of DNA in chromatin isolated from intact nuclei in which lysine-rich or arginine-rich histones were selectively extracted. Measurements of DNA were on nuclei containing 20 to 1200 µg of DNA per ml, the distinctions becoming clear at higher concentrations. Experiments with RNA polymerase showed only moderate increases in template activity upon extraction of histone, although the removal of lysine-rich histone had a greater effect than that of arginine-rich histone. DNA polymerase action on nuclei minus lysine-rich histone achieved high results, exceeding even those on DNA itself.

Toward the end of a recent paper we said, "Since we have not found a report concerning effects of histones on the accessibility of DNA in isolated nuclei to exogenous DNA polymerase, we are presently doing such experiments" (1). The present paper contains such a report. We have prepared calf-thymus nuclei and have from them prepared nuclei minus lysine-rich histone or minus arginine-rich histone. On such nuclei we have measured the template effects of exogenous DNA and RNA polymerases. In all these experiments reference should be made to the changes observed in the fine structure of thymus nuclei from which lysine-rich or arginine-rich histones were extracted. Removal of lysine-rich histone caused the large masses of dense chromatin to be replaced by a network of diffuse fibrils. No change was observed when even more than 50% of the arginine-rich histone was extracted (2). When the template activity of histone-depleted nuclei was measured with DNA polymerase, extraction of lysine-rich histone greatly increased accessibility of the DNA, whereas removal of arginine-rich histone had much less of an effect. In contrast, removal of either class of histone only moderately increased template activity toward RNA polymerase, although extraction of lysine-rich histone resulted in a greater increase. We have already published a study of the action of both DNA and RNA polymerases on intact nuclei in which the incubation with the enzymes was outlined (3), and template activity was found to be very slight.

In previous papers (2, 4) the preparation of chromatin minus lysine-rich and arginine-rich histones has been described. The selective removal of lysine-rich histone from nuclei prepared in sucrose or in 0.01 M citric acid by extraction with 0.10 M citric acid–0.12 M NaCl and of arginine-rich histone with mixtures of alcohol and hydrochloric acid deserves a word of caution. If it is desired to freeze the nuclei, then it is important to wash them with 0.25 M sucrose–0.004 M CaCl₂ before freezing. They then retain their activities with both DNA and RNA polymerase. Otherwise, when frozen they retain only about 40% of their activity. And if nuclei are frozen after being prepared, then it is possible to extract the lysine-rich and arginine-rich histones, but the nuclei retain somewhat less than 40% of their activity.

The Escherichia coli DNA polymerase used in the experiments of Fig. 1 acts on double-stranded DNA and also on single-stranded DNA. Because of the use of 0.10 M citric acid–0.12 M NaCl and of alcohol–HCl for removing histones, it seemed possible that single-stranded DNA was present. Bollum has prepared from thymus a DNA polymerase that acts only on single-stranded DNA (5). We therefore tried the effect of this enzyme (purchased from General Biochemicals, Chagrin Falls, Ohio). It was entirely without action on either nuclei minus lysine-rich or arginine-rich histones and also on DNA prepared from nuclei that had been treated with 0.10 M citric acid–0.12 M NaCl. Thus, the presence of transcribable single-stranded DNA was eliminated as a contributing factor in these experiments.

RESULTS AND DISCUSSION

Fig. 1 gives the results for the action of DNA polymerase on nuclei minus lysine-rich or arginine-rich histones and also at the bottom the action of the enzyme on intact thymus nuclei. Practically all of the lysine-rich histone was removed. Of the arginine-rich histone, between 21 and 51% of the DNA was removed from thymus nuclei. The electrophoretic procedure for analysis of histones has been described (4). Fig. 2 contains the results of the action of RNA polymerase on nuclei from which either of the histones had been removed.

The results on chromatin of nuclei are to be compared with those of More and Paul on extracted thymus chromatin (6). They removed lysine-rich histone with 0.45 M NaCl and measured the effect of E. coli RNA polymerase on the product. The amount of chromatin used is not mentioned. There was no increase over the effect observed in 0.35 M NaCl, when no histone was extracted. These results are entirely different from those we have obtained. They have much in common with those of Spelsberg and Hnilica, also on extracted chromatin (7). Apparently, when chromatin is extracted from the thymus nucleus a change occurs so that the effect of removal of lysine-rich histone is lost.

It is clear from a comparison of Fig. 1 with Fig. 2 that the effect of removal of histones on the template activity with
RNA polymerase as shown in Fig. 2 is relatively slight. The extraction of up to one-half of the arginine-rich histone has not significantly changed the fine structure of the chromatin (2). Removal of lysine-rich histone has a considerably greater effect on the RNA polymerase activity and this can be correlated with the effect on fine structure. Even so, the effects with this enzyme are moderate and in line with what one might expect of the intact nucleus.

In the case of DNA polymerase it is clear that removal of lysine-rich histone has had a most pronounced effect (Fig. 1), as would be expected from what happens during the S phase, when all of the DNA is replicated. It does not, of course, indicate that during the S phase all of the lysine-rich histone is displaced, but even a reduction of this histone by 1/4 could be adequate, or possibly the phosphorylation of the lysine-rich histone would suffice (8, 9). Even the curve at the base of Fig. 1, showing what happens to intact nuclei, is low, but distinctly higher than the curve at the bottom of Fig. 2. The various curves on Fig. 1 show that it would not require much of a change in the lysine-rich histone to enable the cell to replicate its DNA once the DNA polymerase was formed. The big increase in template activity due to removal of lysine-rich histone is in line with the large change in fine structure of chromatin caused by extraction of this histone. And the slight increase in template activity of DNA polymerase caused by 21-51% removal of arginine-rich histone is to be expected from the absence of change noted in the fine structure of chromatin associated with extraction of up to 51% of this histone. These results are closely parallel to those obtained when DNase rather than DNA polymerase is used as a probe of chromatin structure (4). Further experiments involving the template activity of histone-depleted nuclei to which histones have been added, confirm the present results (manuscript in preparation).

A very striking change noted in Fig. 1 is the decline in template activity of DNA after 200 μg. All that can be said is that "it is not uncommon to find that, while the Michaelis law is obeyed at lower substrate concentrations, the velocity
falls off again at high concentrations" (10). However, even if DNA template activity reached a plateau at the level of 200 μg of DNA per ml, the conclusions drawn from Fig. 1 would be the same. And of course one must remember that in the nucleus itself the DNA concentration greatly exceeds the highest level we have used in these experiments. Thus, the trend of our observations at “high” concentrations of DNA would be expected to reflect the true situation in the functioning nucleus.

We thank Miss Ellen Hein for her assistance. This work was supported in part by a grant from the New York Heart Association.