THE RELATIVE ABILITY OF RECONSTITUTED NUCLEOHISTONES TO ALLOW DNA-DEPENDENT RNA SYNTHESIS

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Huang and Bonner (1962) have recently demonstrated in a system derived from pea embryo chromatin that DNA fully complexed with histone is inactive in the support of DNA dependent RNA synthesis. Allfrey, Littau, and Mirsky (1962), using isolated calf thymus nuclei have also shown that partial removal of histone by trypsin treatment resulted in increased RNA synthesis. This communication is concerned with the relative ability of reconstituted DNA histone complexes to permit DNA dependent RNA synthesis.

Materials and Methods

Calf thymus DNA was isolated by the method of Kay, Simmonds and Dounce (1952).

The total histones of thymus nuclei were prepared by acid extraction of the isolated nuclei with cold 0.1N HCl. The extract was dialysed briefly against water and the histone chlorides precipitated with 12 volumes of cold acetone. The very lysine rich histones were prepared by extraction of the total histones with 5% perchloric acid (PCA) and the slightly lysine rich and arginine rich histones were isolated by chromatography on carboxymethylcellulose (Johns, Phillips, Simson and Butler, 1960). Reconsti-

tuted nucleohistone was prepared by mixing histone and DNA in a weight ratio of about 2.5:1 in a solution of high ionic strength 2.5M NaCl) and slowly lowering the ionic strength by dialysis against 0.05M Tris/HCl, pH 7.9, 0.005M $MgCl_2$. With the very lysine rich histone this method gave a very sticky DNP preparation. Gradual dilution of the high ionic strength mixture with constant stirring however gave a suspension of nucleohistone which was amenable to further handling.

The nucleohistones were washed three times by suspension in the Tris-Mg buffer and finally resuspended in the reaction mixture buffer (0.04M Tris/HCl, pH 7.9, 0.001M $MnCl_2$, 0.004M $MgCl_2$, 0.012M β-mercaptoethanol).

RNA-polymerase was isolated from E. coli B by the method of Chamberlin and Berg (1962). The assay used measured the incorporation of C^{14} from labelled uridine triphosphate into acid insoluble form. The incubation mixture contained 0.50 ml of the reaction mixture buffer, 150 mμ mole each of ATP, GTP and GTP, 0.025 μC UTP-2-C^{14} (Sp. activity 24.6 μC/mg), 25 μg of purified enzyme and DNA or the nucleohistone. After incubation at 37°C for 10 min. the reaction mixture was chilled, 0.5 mg bovine γ-globulin added as a carrier followed by 5.0 ml of cold 5% PCA. The precipitate was filtered off on a millipore membrane and washed four times with 5 ml portions of PCA. The membrane was then stuck to an aluminium planchette, dried at 60°C and counted in a gas flow counter.

Results

In the presence of 0.10 mg DNA, the assay was proportional to the amount of enzyme added in the range 2-40 μg enzyme. With the purified enzyme, RNA synthesis was dependent on the addition of DNA and the four nucleoside triphosphates (Chamberlin et al., 1962).

In contrast to DNA, nucleohistone is completely insoluble in the assay system and it is therefore not possible to compare directly their ability to function as templates for RNA synthesis. However, by complexing DNA with a basic protein (lysozyme) an insoluble nucleoprotein preparation could be obtained the "priming" efficiency of which could then be compared to that of the nucleohistones.

Table 1 shows the results of an experiment in which the rleative efficiences of reconstituted nucleohistone (using unfractionated histone) and reconstituted nucleolysozyme for allowing RNA synthesis are compared. This experiment shows that DNA fully complexed with lysozyme allows RNA synthesis to proceed at about 70% of the rate obtained using an equivalent amount of free DNA. On the other hand, DNA bound to histone is completely inactive in this respect.

Table 1

Relative efficiency of reconstituted nucleohistone and nucleo-lysozyme in "priming" RNA synthesis

Components	Relative incorporation of UTP-2-C14 (c.p.m.)
Complete System minus DNA or DNP	0
" " plus 100 µl nucleohistone suspension*	1.8
" " " 75 µl " "	1.4
" " " 50 µl " "	2.0
" " " 20 µl " "	0
" " " 10 µl " "	2.5
" " " 150 µl nucleolysozyme suspension**	2,101.
" " " 100 µl " "	1,932.
" " " 50 µl " "	1,726.
" " " 25 µl " "	1,381.
" " " 15 µl " "	1,222.
" " " 37 µg free DNA	2,602.

* containing 44 µg bound DNA/100 µl
** containing 28 µg bound DNA/100 µl

Table 2 presents the results of experiments in which the "priming" efficiences of complexes of DNA with different histone fractions are compared.

Table 2

Relative "priming" efficiency of complexes of DNA with different histone fractions

Components	Relative incorporation of UTP-2-C^{14} (c.p.m.)
Complete System minus primer	0
" " plus 200 µl nucleohistone A suspension	1,537
" " " 100 µl " " "	918
" " " 50 µl " " "	634
" " " 25 µl " " "	348
" " " 200 µl nucleohistone B "	20.4
" " " 100 µl " " "	5.5
" " " 50 µl " " "	0.3
" " " 25 µl " " "	1.4
" " " 200 µl nucleohistone C "	0.9
" " " 100 µl " " "	2.1
" " " 50 µl " " "	1.2
" " " 25 µl " " "	0.4
" " " 100 µl nucleolysozyme suspension	1,097

Nucleohistone A contains the very lysine rich histone
" B " " " slightly lysine rich histone
" C " " " arginine rich histone

All nucleoprotein suspensions contain about 40 µg bound DNA/100 µl

It is seen that DNA complexed with either the arginine rich or slightly lysine rich histones is almost completely inactive in the support of DNA dependent RNA synthesis. On the other hand, the complex with the very lysine rich histone allows RNA synthesis to proceed at a rate comparable to that obtained with the DNA-lysozyme complex.

The results presented show that the association of DNA with specific histones can considerably modify the ability of the former to function as a template for RNA synthesis. This may have a role in the intact cell of controlling the synthesis of messenger RNA on specific regions of the DNA genome and hence the expression of particular genes.

References

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