EFFECT OF THE SUPRANUCLEOSOMAL ORGANIZATION OF CHROMATIN ON HISTONE – DNA RELATIONS

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The hierarchical principle of the structural organization of chromatin and chromosomes is widely accepted [6, 7]. It is natural to expect that the formation of higher structural levels, accompanied by a further increase in the compactness of DNA, must change relations between DNA and the proteins bound with it. It is not clear, however, how great these changes must be in order to give rise to fundamental changes in genome function. In an attempt to examine this problem we decided to study whether an increase in the degree of dispersion of chromatin, disturbing more especially the larger structural domains and, in particular, DNA loops, affects the strength of binding of histones with DNA. To estimate this strength, the phenomenon of competition of histones for DNA, first described by Ashmarin and Muratchaeva [1], was used. The writers showed previously [3, 4] that one result of this phenomenon is replacement of some of the histones of chromatin by the most competitative fractions of total histone added to it in excess.

The starting point of the investigation was the assumption (determining how the effectiveness of displacement of its own histones from chromatin by histone added to it in excess depends on the degree of dispersion of the chromatin) can shed light on the effect of the higher structural formations of chromatin (supranucleosomal) on histone–DNA interaction.

EXPERIMENTAL METHOD

Chromatin was obtained as described previously [4] from calf thymocytes and transferred into 0.15 M NaCl + 0.7 mM Na-phosphate buffer, pH 7.0. Total histone of chromatin was obtained by extraction with 0.4 N HCl (30 min, 0°C). The chromatin suspension was incubated with the added total histone for 48 h at 4°C. Residues after centrifugation of the mixtures for 30 min at 40,000 rpm on an L2-65B centrifuge (Beckman, USA, 40.3 rotor) were used to analyze proteins bound with DNA, by electrophoresis [8]. Densitometry of the gels stained after electrophoresis with Coomassie blue R-250 was carried out on a "Gilford" spectrophotometer at 580 nm. Other details of the experimental method were described previously [4].

EXPERIMENTAL RESULTS

Densitograms of the gels after electrophoresis of proteins remaining bound with DNA after incubation of three chromatin preparations with different quantities of excess total histone in medium with near-physiological ionic strength (0.15 M NaCl + 0.7 mM Na-phosphate buffer, pH 7.0) are shown in Fig. 1 (a, b, c). Addition of total histone to the chromatin in a quantity equivalent by weight to that already contained in it, or in a higher quantity, led to complete displacement of histone H1 from DNA. All the nucleosomes thus lose an H1 molecule, i.e., all nucleosomes are stereochemically accessible for molecules of histone added to the chromatin and competing for DNA in all three preparations. However, histones H2A and H2B in the first chromatin preparation virtually are not displaced if the ratio of added total histone to DNA is 200:1. In the second preparation histone H2A and H2B are partially displaced when the above ratio reaches 20:1 or even 5:1. In the third preparation, treated with ultrasound in order to increase the degree of dispersion, with added histone in a 20-fold excess relative to DNA, far less of the H2A and H2B histones remained bound to DNA than in the second preparation under the same conditions.

An increase of dispersion of the chromatin preparation (dispersion was higher in the second preparation than in the first, evidently on account of more vigorous mechanical dispersion) thus leads to displacement of

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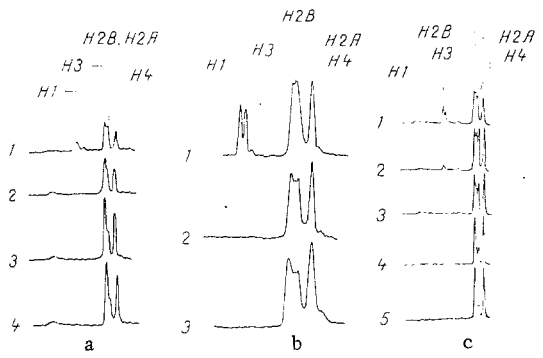

Fig. 1. Densitograms of gels after electrophoresis of histones in complex with DNA after incubation of chromatin with added total histone. Ratios of added histone to chromatin DNA. a: 1) 0, 2) 1, 3) 20, 4) 200; b: 1) 0, 2) 5, 3) 20; c: 1) 0, 2) 0.8, 3) 2, 4) 20, 5) 40.

histones of the nucleosome nuclei H2A and H2B from DNA on account of competition by fractions H3 and H4. This can be interpreted as weakening of interaction with DNA of histones H2A and H2B with an increase in dispersion of the chromatin.

What is the cause of this phenomenon? Since the increase in dispersion of chromatin is realized by a decrease in length of the DNA molecules in its fibrils, the number of disturbed terminal nucleosomes, in which interaction of histones with DNA may be weakened on account of repacking, may be increased at the same time. The molecular weight of DNA in the sonicated preparation of chromatin was $5 \cdot 10^6$ daltons (data of viscometry). If the molecular weight of DNA per nucleosome is 600 daltons · 200 pairs = 132,000 daltons, on average each fibril in the sonicated chromatin preparation will contain about 37 nucleosomes. In that case, even if each of the terminal nucleosomes is disturbed, the histones contained in it will account for only 6%. Differences in competitive displacement of histones H2A and H2B from the first and third chromatin preparations cannot be explained by weakening of the bonds joining histones to DNA in the terminal nucleosomes, for the proportion of such histones is small in both preparations. The difference in the quantity of displaced histones H2A and H2B in these preparations, however, is fundamental in character. Displacement of histones H2A and H2B from the first preparation could not be recorded even if added histone was present in a 200-fold excess, but for the third preparation a 20-fold excess of total protein was sufficient to cause displacement of the greater part of the histones H2A and H2B.

These results can be understood on the basis of the view that organization of DNA in the supranucleosomal structures leads to a sharp increase in the intensity of interaction of DNA with histones of the nucleosomal nuclei. In particular, the formation of supercoiled loops in the chromosomes may prevent dissociation of histones H2A and H2B, making them stereochemically inaccessible, by contrast with H1, for free histones competing for DNA. Destruction of such loops makes competitive displacement of histones H2A and H2B possible. This conclusion is in agreement with the view [10] that bonds joining proteins with DNA are weakened after relaxation of the circular DNA of the minichromosomes of virus SV-40 which, in the opinion of the authors cited, explains the decrease in the protein content on relaxed DNA molecules. Our conclusion is also supported by the fact that in a mixture of artificial nucleohistone with total histone, with histone in a 20-fold excess over DNA, only histones H3 and H4 remain in the form of a complex with DNA [2]. However, histones H2A and H2B are not completely removed from sonicated chromatin even if total histone is present in a 40-fold excess. This can be attributed to the very strong interaction of histones of nucleosomal nuclei with DNA in supercoiled loops, remaining after sonication of the chromatin. This possibility is confirmed by the fact that on average the DNA molecules from sonicated chromatin contain about 8000 base pairs, which is close to the dimensions of the small loops discovered in chromatin (10,000 base pairs [6]), allowing for the polydispersed state of DNA. In chromatin preparations isolated in medium with near-physiological ionic strength, mol. wt. of DNA, according to our viscometric data, is $25 \cdot 10^6$–$65 \cdot 10^6$ daltons, i.e., 38,000–98,000 base pairs. The loops may be completely preserved in these preparations, and fragmented loops are removed when the chromatin suspension is washed. This evidently helps to preserve histones H2A and H2B on DNA in the presence of a 200-fold excess of added total histone.

Competitive displacement of histones from chromatin by added histones is based on the dynamic character of histone–DNA relations in the chromatin, which has recently been confirmed by experiments similar to those conducted by the present writers previously [2-4], to analyze exchange of chromatin, fragmented by micrococcal nuclease, and exogenous histones [9]. In this connection it can be tentatively suggested that supercooling of DNA loops in the cell is a mechanism of conversion of dynamic DNA–histone relations into static or, in other words, a mechanism of blocking of functional activity of DNA. If, in fact, realization of transcription requires displacement of histones by RNA-polymerase [5], to enable access of this enzyme to the histones it is possible to use destruction of the DNA loops, just as for displacement of H2A and H2B by added histones. The conclusion is in agreement with the view that relaxation of DNA of the minichromosomes of SV-40 is necessary for activation of transcription [10].

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INTERACTION OF MOUSE LYMPH NODE AND SPLENIC LYMPHOCYTES ON INACTIVATION OF ALLOGENEIC HEMATOPOIETIC STEM CELLS

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The role of and interaction between subpopulations of T lymphocytes in the phenomenon of inactivation of nonsyngeneic hematopoietic stem cells (HSC), discovered by Petrov and Seslavina in 1967 [7], have been investigated on several occasions [3, 4, 8]. It has been shown that T lymphocytes are effector cells in the inactivation reaction, and comparative characteristics of the inactivating activity of different subpopulations of T lymphocytes, and so on, have been obtained. In a study of interaction between T-lymphocytes subpopulations from the spleen and lymph nodes (LN) of T mice with an artifically created B-cell deficiency, it was concluded that the spleen contains suppressor cells, protecting the stem cells against the inactivating action of allogeneic T-lymphocytes [3]. Meanwhile the attempt to study interaction between intact splenic and LN lymphocytes in this phenomenon proved unsuccessful at this stage because of the complex character of interaction. Later work showed that an important role in the inactivation phenomenon is played not only by T lymphocytes, but also by B lymphocytes of LN. While not possessing an effector function, they have a regulatory influence, reducing or enhancing the inactivating activity of T lymphocytes of LN, depending on the quantitative ratio between them [2, 6].

The aim of this investigation was to study the principles governing interaction of intact LN and splenic lymphocytes from mice during inactivation and discovery of the role of B lymphocytes in this interaction.

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