CHANGES IN PROTEIN COMPOSITION OF CHROMATIN IN THE PRESENCE OF EXTRA TOTAL HISTONE

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In several laboratories analysis of the reaction of cell nuclei and DNP extracts from animal tissues to added histones is used as an original approach to the study of the structural organization of chromatin [1, 3, 8, 10-12]. The idea behind these investigations was the suggestion that chromatin can bind a strictly limited quantity of repressor histone molecules either because it contains active genes unrepressed by histones or because individual phosphate groups of DNA of all genes of the chromosomes, by virtue or some as yet unexplained necessity, remain free from binding with protein amino groups of chromatin. However, data so far obtained have led some workers to question the basic assumption and to suggest that histones bind, not with DNA of chromatin, but with its protein component [1, 10, 11]. Later work [3, 12] has not resolved this contradiction, but the conclusion has been drawn that the extra histones in the chromatin preparations which they used were bound with DNA.

In the works cited above [1, 3, 8, 10-12] no attempt was made to analyze the possibility that interrelations between macromolecular components of chromatin could be reorganized by the bound histones. It is not known whether individual fractions of total chromatin histone show any preference for binding with chromatin, such as they do when they bind with DNA [9]. The question of how the protein composition of chromatin changes on the addition of an excess of total histone has not been studied, although it is important to know this in the light of views on the existence of an extrachromosomal pool of histones in cell nuclei [5, 7].

In the investigation described below an attempt was made to make good these gaps in the modern understanding of chromatin.

EXPERIMENTAL METHOD

Chromatin preparations were isolated from calf thymus [13]. Total histone was extracted from the chromatin preparations with 0.4 N HCl. The DNA concentration was measured by Spirin's method [2] and the protein by Lowry's method [6], using for calibration a solution of bovine serum albumin (from Merck, West Germany), made up on the basis of an extinction coefficient $E^{1\%}_{278\ nm} = 6.67$. The chromatin preparations (C_{DNA} = 100 μg/ml) were mixed with an equal volume (4 ml) of the histone solutions in different concentrations in 0.7 mM Na-phosphate buffer, pH 7.0. The mixtures were incubated for 12 h at 4°C and centrifuged for 8 h on an L-2-65B centrifuge (from Beckman, USA), with 40.3 rotor, at 40,000 rpm. The residues were washed with distilled water to remove traces of free histone and their protein composition was analyzed by electrophoresis [4], after preliminary determination of the protein/DNA ratio.

EXPERIMENTAL RESULTS

Protein/DNA ratios were obtained by analysis of DNP complexes, formed by addition of different quantities of total protein to the chromatin preparations, and sedimented by centrifugation. As Table 1 shows, the chromatin completely bound all total histone fractions if the histone was added in an amount equivalent by weight relative to DNA. If twice the quantity of total histone was added, a certain part of it did not take part in complex formation with chromatin. This implies saturation of the functional groups of the chromatin that are capable of binding histones. However, on the addition of further total histone to the chromatin, a nucleoprotein complex with a higher protein content was formed.

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TABLE 1. Characteristics of DNP Complexes Formed in Mixtures of Chromatin and Total Histone

Protein/DNA in DNP complexes	Added histone/DNA of chromatin											
	0	1	2	3	4	5	6	8	10	20	30	40
I	1,30	2,32	2,46	3,10	3,27	2,96	3,24	3,03	2,95	2'44	2,44	2,23
II	1,22	2,29	2,79	2,86	2,96	3,10	3,18	3,21	2,80	2,82	2,5	2,46

Legend. Experiments carried out on chromatin preparations were obtained from the thymus of different calves (protein/DNA ratios 1.30 and 1.22); different preparations of total histone were used.

This important aspect of histone binding with chromatin failed to engage the attention of previous investigators. Nevertheless, the cause of the phenomenon may naturally be sought in the fact that saturated nucleoprotein complexes formed in mixtures of chromatin with different quantities of total histone differ in their protein composition. If chromatin proteins and, in particular, histones have different values of the ratio of weight of the protein molecule (M) to the number of functional groups (N) capable of binding with DNA (and this actually is the case [9]), saturated nucleoprotein complexes differing in protein composition will be characterized by different values of the protein/DNA ratio, which can be expressed as follows:

$$\frac{\text{Protein}}{\text{DNA}} = \frac{M_{\text{protein}}/N}{M_{\text{nucleotide}}}.$$

The results of electrophoretic analysis of the residues obtained after centrifugation of mixtures of chromatin solutions of equal concentration with equal volumes of solutions of total histone in different concentrations are given in Fig. 1. They show that if total histone was added to chromatin in twice the equivalent quantity relative to DNA, the chromatin lost its histone H1. If a sufficiently large quantity of total histone was added to the chromatin, a nucleoprotein complex containing only histones H3 and H4 was formed in the mixture. These facts, established for the first time, compel a new look at the results of previous investigations along the same lines.

A complex of histone with extra histones is known to contain histones that are less firmly bound than the histones of chromatin proper [1, 11]. This suggested that histones added to chromatin are bound, not with phosphate groups of DNA, but with carboxyl groups of proteins, and that the extra histones are themselves weakly bound [1, 11]. Phillips [11] showed that histones H2A and H2B are extracted from the total histone–chromatin complex on the addition of an amount of HCl which cannot dissociate even the least firmly bound histone H1 in the original chromatin. It will be clear from Fig. 1 that an increase in competition between histones for DNA with an increase in their concentration in the chromatin solution leads to complete displacement from the chromatin initially of histone H1, and later of histones H2A and H2B. It might be supposed that displacement of the intrinsic histones of chromatin can take place only through the action of fractions H3 and H4 from the added histone, and that the remaining fractions are less firmly bound than the intrinsic histone H1 of chromatin. In that case, on addition of a quantity of total protein sufficient to displace all the histone H1 from its complex with DNA to the chromatin (ratio of added histones to DNA 3:1), all the fractions H1, H2B, and H2A of the added histone ought to be present in the supernatant. But this did not occur. As Table 1 shows, 28-32% of protein remained in the supernatant of this chromatin–histone mixture. This is much less than the expected quantity of protein if the previous assumption was fulfilled. Accordingly it can be categorically asserted that the histones added to the chromatin are not attached by labile bonds to chromatin which has preserved its normal structure, as was considered previously [1, 10, 11], but they reorganize the DNA–histone relations in the chromatin, by weakening the bonds between them. As a result the histones begin to dissociate from DNA under milder conditions, although the order of their dissociation remains the same [11].

It is also clear that histones added to chromatin bind with DNA. Otherwise they could not have displaced the intrinsic histones of the chromatin. This conclusion is supported by data of Clark and Felsenfeld [3], obtained by titrating phosphate groups of DNA in chromatin–histone complexes with $^{54}Mn^{++}$ ions, and data showing reduced accessibility of chromatin DNA to DNases in the presence of extra histones [3, 8]. The results obtained by Paul and More [10], who used toluidine blue to titrate DNA phosphate groups in chromatin, are an exception: They concluded that addition of histones to chromatin does not reduce the number of phosphate groups accessible for binding with the dye, and for that reason the histones bind exclusively with chromatin protein. It has been pointed out [3] that the conclusion given above [10] may be incorrect because of the diffi-

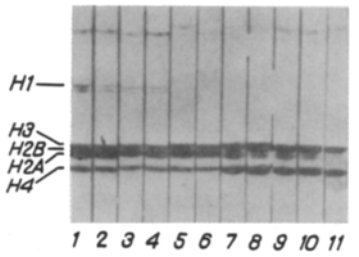

Fig. 1. Electrophoretic analysis of residues obtained by centrifugation of chromatin – total histone mixtures in 0.7 mM Na-phosphate buffer, pH 7.0. Histone/chromatin DNA ratio in mixtures: 1) 0; 2) 0.5; 3) 1; 4) 2; 5) 3; 6) 4; 7) 6; 8) 10; 9) 20; 10) 30; 11) 40.

culty of interpreting quantitatively the results of dye experiments because of the presence of two types of binding sites for dyes on DNA. There is yet another possible incorrect interpretation of these experiments, which is that the dye could have greater competitiveness in binding with DNA than with Mn^{++}. The dye could therefore displace from DNA some histone amino groups on sites weakly bound with DNA, from which the mistaken conclusion could be drawn that the histones were bound not with DNA, but with the chromatin protein. The greater competitiveness of the dye than of Mn^{++} for binding with phosphates may be due to the fact that, as a surface-active substance, the dye affects interhistone interactions which, as the present writers have shown [9], make an important contribution to the strength of the bonds of histones with DNA.

The data described above on displacement of intrinsic histones of chromatin and on structural reorganization of chromatin by histones added to it are of great importance for weighing up the data in the literature on the existence of an extrachromosomal histone pool in cell nuclei [5, 7]. The possibility cannot be ruled out that these histones exist in the nucleus in a deposited state, inaccessible for binding with DNA.

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