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HISTONE COMPETITION FOR DNA AND ITS POSSIBLE SIGNIFICANCE FOR THE CHROMATIN SELF-ASSEMBLY

The histone competition for DNA has been described by J o h n s and B u t l e r [16]. However, we know only one work (A s h m a r i n & M u r a t c h a e v a [1]) on the importance of this phenomenon for histone binding to DNA. In both the papers as well as in our previous reports (P a p o n o v et al. [32-34] the histone competition for DNA is used only as an experimental method for analysis of relative histone affinity for DNA. Nobody has analysed the possibility of histone competition for DNA in cell and naturally the question of its biological consequences has not been posed. To reveal the above possibility it is necessary, in model systems, to determine the whole histone/DNA ratios at which the histone competition for DNA arises. It is also important to study the selectivity of the competition with respect to individual histone fractions when all the histones can compete for DNA being specifically associated with each other.

In the course of the studies performed in our laboratory DNA was isolated from calf thymus as previously described (P a p o n o v et al. [32, 33]) and sonicated with an MSE ultrasonic disintegrator ($M_{r\ DNA} = 1 \times 10^6$). DNA was quantified spectrophotometrically according to S p i r i n [46]. Histones were extracted with 0.4 N HCl from calf thymus chromatin, which was isolated by the procedure of Z u b a y & D o t y [56]. Protein was determined according to L o w r y et al. [26] with whole thymus histone as a standard. Concentration of histone stock solution was determined by measuring absorbance at 230 nm, $A^{1\%}_{1cm}$ at 230 nm being taken as 42.5 (O h l e n b u s c h et al. [31]).

Whole histone-DNA mixtures were prepared by direct addition of histone solutions of various concentrations to fixed amounts of DNA in two media: (a) in solution of physiological ionic strength, (b) in 2M NaCl with subsequent salt gradient dialysis in the same solution of physiological ionic strength. DNA con-

centration in all mixtures was 0.1 mg/ml. After 16 h incubation in 0.15 M NaCl/0.7 mM sodium phosphate buffer (pH 7.0) at 2 ÷ 4°C DNA-histone mixtures were centrifuged for 6 h at 114,000 g (40,000 revs/min in 40.3 rotor). Histones of the supernatants and the pellets were resolved by electrophoresis on SDS gels according to L a e m m l i's procedure (L a e m m l i [22]).

In the first stage of the studies we carried out the analysis of competitive histone binding to DNA.

Figure 1A shows that after centrifugation of whole histone-DNA mixtures at histone/DNA ratios less than or equal to 1 the histones are absent in the supernatants obtained. In other words, the histones completely bind to DNA and appear in the sediments. Some DNA of the above mixtures remains in the supernatants being free from histones. This finding agrees with results of R u b i n & M o u d r i a n a k i s [38] and it is explained by co-operative binding of histones to DNA at the physiological ionic strength.

After centrifugation of whole histone-DNA mixtures at histone/DNA ratios over or equal to 1.1, DNA is absent in the supernatants. That is consistent with the data of F r a n c o et al. [8]. However, the supernatants contain histones. This suggests the existence of histone competition for DNA in the mixtures with whole histone/DNA ratio greater than 1.

Figure 1B shows histone/DNA ratios in sediments obtained after centrifuging the whole histone-DNA mixtures. We can see that protein content in the nucleohistones assembling in the mixtures depends onthe input histone/DNA ratio. However, starting from a certain stage, a further strengthening of the histone competition for DNA (i.e. increasing the histone/DNA ration in the mixtures) does not practically affect the histone/DNA ratio of the nucleohistones formed in the mixtures. These saturated DNA--histone complexes had in various experiments the following histone/DNA ratios: 1.46, 1.43, 1.62, 1.37, 1.36. It should be emphasised that the above complexes were formed in the mixtures at a 50-fold excess of the whole histone over DNA.

The dependence of protein/DNA ratio of nucleohistones formed in the DNA-histone mixtures on the input ratio of the components was previously reported (R u b i n & M o u d r i a n a k i s [38]; L a p e y r e & B e k h o r [23]). It was also noted that DNA cannot bind more than a certain amount of histone in the mixtures of DNA with individual histone fractions (P a u l & M o-

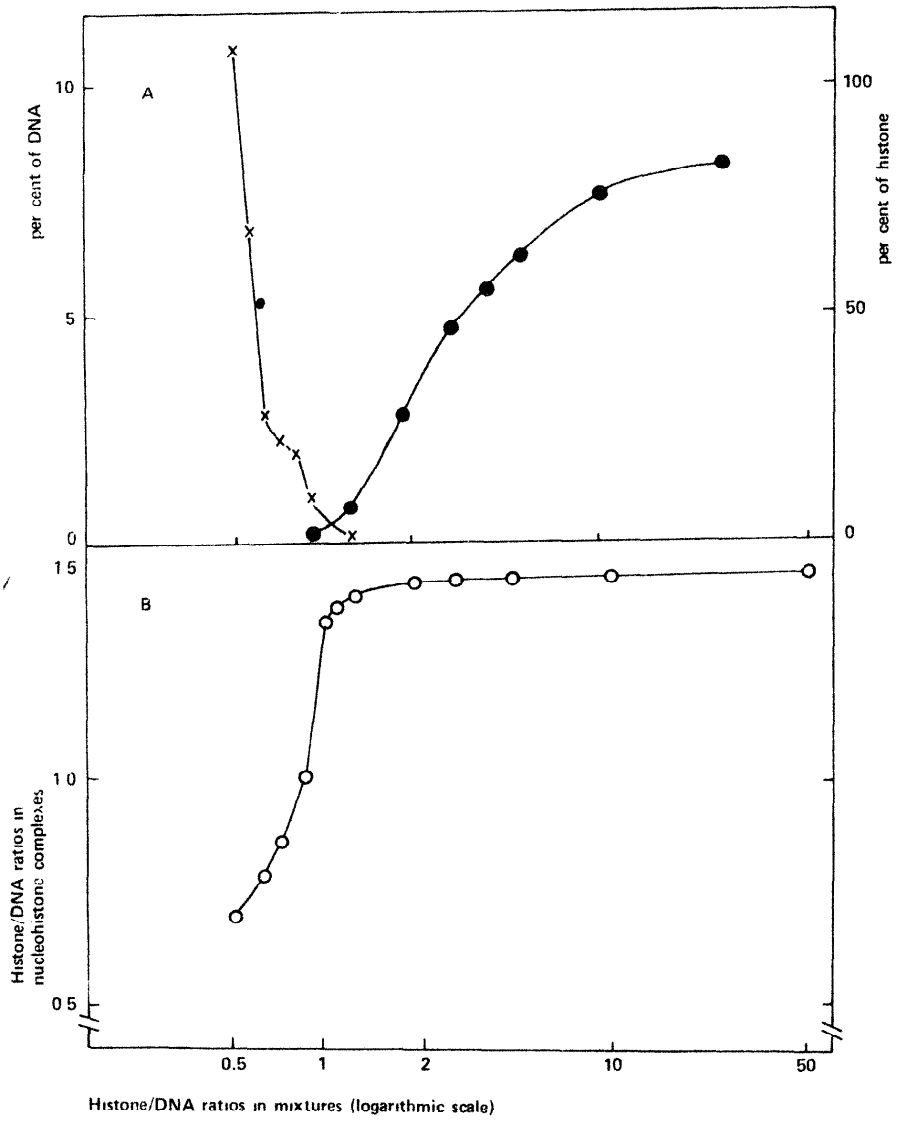

Fig. 1A. Recovery of DNA (x— x) and histones (—— · ——) in supernatants after centrifugation of whole histone-DNA mixtures in the medium of physiological ionic strength (0.15 M NaCl, 0.7 mM sodium phosphate buffer, pH 7.0)

Fig. 1B. The effect of whole histone/DNA ratios in initial mixtures on composition of nucleohistone formed in the medium of the physiological ionic strength

r e [36]) as well as in those of DNA with the whole histone (V o o r d o u w et al. [51]). However, the composition of histone fractions of the nucleohistones assembling in whole histone-DNA mixtures is usually not analysed. We could find the relevant information only in the work of J o h n s & B u t l e r [16] who showed that the nucleohistone containing only the histone H3 is formed at a whole histone/DNA ratio of 4.

Electrophoretic analysis of histones in sediments of the mixtures showed that H1-depleted and H1-free nucleohistones are

formed in initial mixtures at histone/DNA ratios less than 1.5 and greater or equal to 1.5 respectively (Fig. 2). Thus, rather a small excess of the whole histone over DNA in their mixtures is capable of complete suppression of H1 binding to DNA at physiological ionic strength.

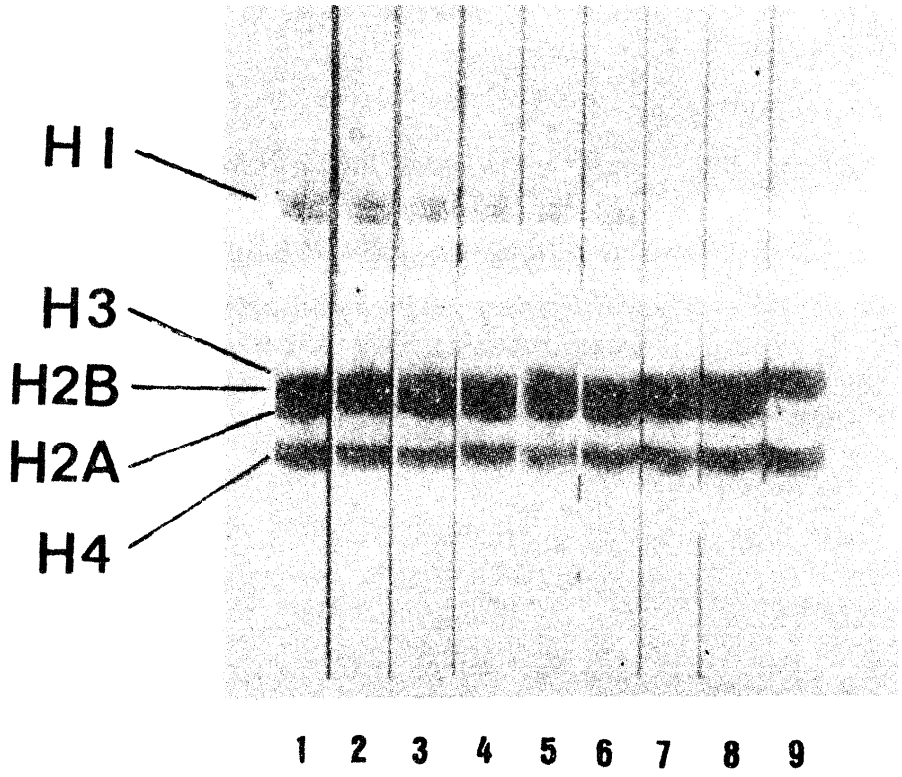

Fig. 2. Electrophoretic analysis of histones in pellets obtained by centrifugation of whole histone-DNA mixtures

Histone/DNA ratios in initial mixtures (w/w): (1) 0.9; (2) 1: (3) 1.1; (4) 1.2; (5) 1.3; (6) 1.4; (7) 1.5; (8) 1.9; (9) 20

The analysis of supernatants of the DNA-histone mixtures showed that the binding of histone H1 to DNA is selectively suppressed at whole histone/DNA ratios between 1.1 and 1.4 (see Fig. 3). Other histone fractions at these histone/DNA ratios completely bind to DNA because they were not found in the supernatants. When the histone/DNA ratios were greater or equal to 1.5 other histone fractions appeared in the supernatants, i.e. competition between the histones of nucleosome cores H2A, H2B, H3, H4 for DNA started. As result of this competition the nucleohistone containing only histones H3 and H4 was formed at high histone/DNA ratios (see Fig. 2).

A histone/DNA ratio did not differ for saturated nucleohistones containing either H3, H4, H2A and H2B or only H3 and H4 (Fig. 1B). This allows us to conclude that the sizes of the

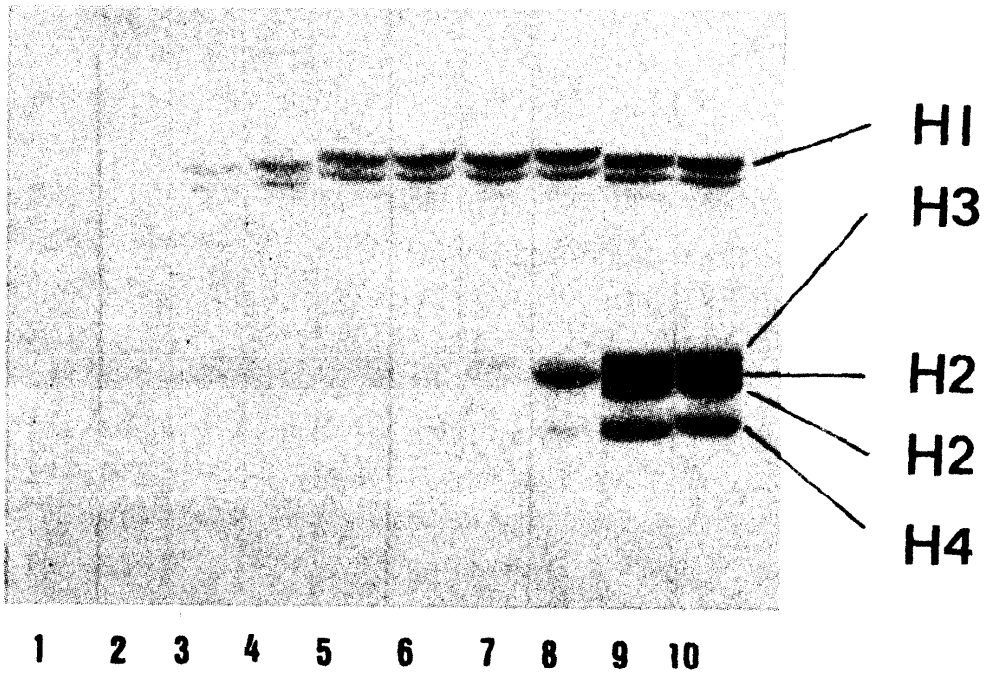

Fig. 3. Electrophoretic analysis of histones remaining in supernatants after centrifugation of whole histone-DNA mixtures
Histone/DNA ratios in initial mixtures (w/w): (1) 0.9, (2) 1; (3) 1.1; (4) 1.2; (5) 1.3; (6) 1.4; (7) 1.5; (8) 1.7; (9) 1.8; (10) 1.9

DNA regions occupied with the pairs H2A + H2B and H3 + H4 are practically equal. On the other hand, as the histone/DNA ratio in nucleohistone containing H1 is less than that in H1-free nucleohistone, we can conclude that this histone or at least a part of its molecule is located between the DNA regions occupied by core histones but not along these regions. This conclusion supports a widespread conception (F e l s e n f e l d [7]).

The main result of the experiments described is that a minimal mass excess of whole histone over DNA in their mixture leads to a self-assembly of nucleohistone depleted in H1 but containing all other histones in the same relative amounts as in the whole histone-DNA mixture.

Further we investigated - histone competition for DNA and performed studies on chromatin assembly.

Many publications are known in which nucleohistones were reconstituted at histone/DNA ratios significantly greater than unity (R u b i n & M o u d r i a n a k i s [38]; Z i m m e r m a n & L e v i n [55]; S t e i n et al. [43]; B e k h o r & F e l d-

man [2]; Lapeyre & Bekhor [23]; Hsiang & Cole [13]; Voordouw et al. [51]; Steinmetz et al. [47]; Weihe et al. [52]; Laskey et al. [25]; Kolchinsky et al. [18]; Stein [44]; Washakidze et al. [50]). All available biochemical and biophysical assays were used to test the fidelity of reconstitution of those complexes but no histone composition of the nucleohistones was analyzed. The authors quoted above seem to believe that all the histone fractions bind to DNA at any excess of total histone over DNA, because the histone/DNA ratios used in the studies were as high as 8 (Voordouw et al. [51]; Vashakidze et al. [50]). Inasmuch as the nucleohistones, in many cited papers, were prepared by salt gradient dialysis from a high salt we should emphasize that selective binding of histones to DNA at histone excess over DNA was also found by us after salt gradient dialysis of their mixtures from 2M NaCl in final physiological ionic strength.

This fact is not unexpected, because three groups of histones H3 + H4, H2A + H2B and H1 are known to bind with DNA at different salt concentrations: 1.2-1.0; 0.8-0.5; < 0.5 respectively (Burton et al. [5]; Wilhelm et al. [53]). Therefore, if the histones H3 and H4 are capable of saturating DNA, no other histones with less affinities for DNA will bind with DNA at a subsequent lowering of ionic strength. It is much more surprising that the selective binding of histones to DNA occurs at a direct mixing of the total histone with DNA in the medium of physiological ionic strength, where all the histone fractions may be in principle attached to DNA. This phenomenon may be explained only by histone competition for DNA.

It is obvious that the histone competition can occur only when the number of histone molecules in the mixture is greater than that of nucleic acid binding sites for histones. According to our findings the above situation takes place at a total histone to DNA weight ratio greater than unity. We can conclude that the total histone saturates the DNA binding sites at the histone to DNA ratio equal to unity. The core histones H3, H4, H2A and H2B do this, however, at a greater histone to DNA ratio (Figs 1 and 2).

This observation is consistent with literature data on stoichiometry of histone binding with DNA if to take into account

that histone H1 is selectively competed out in conditions of a rather low excess of total histone over DNA irrespective of the method of nucleohistone reconstitution. The highest histone to DNA ratio that V o o r d o u w et al. [51] had achieved by reconstituting complexes of four core histones and Col E_1-plasmid DNA was 2.4. J o h n s and B u t l e r [16] obtained the nucleohistone with a histone to DNA ratio of 2.3 by direct mixing of a 4-fold excess of the total histone with the calf thymus DNA in 0.14 M NaCl. In conditions of a 3-4-fold excess of the total histone and of four core histones over DNA the nucleohistones were obtained by salt gradient dialysis with the histone to DNA ratios of 1.7 (K o l c h i n s k y et al. [18]) and 1.86 (T a t c h e l l & V a n H o l d e [49]) respectively. B e k h o r and coworkers obtained the nucleohistones with histone to DNA ratios of 1.5 to 1.8 (B e k h o r & F e l d m a n [2]) and 4.5 (L a p e y r e & B e k h o r [23]) by reconstituting complexes of total histone and DNA respectively in a medium containing 5M urea and in that of physiological ionic strength without urea. S h i h & B o n n e r [42] also noted that stoichiometric ratio was decreased by use of urea in the process of reconstitution of complexes between DNA and separate histone fractions.

We believe in accordance with other authors (S h i h & B o n n e r [42]; K o r n b e r g & T h o m a s [20]) that nonspecific histone aggregation may be the cause of anomalous stoichiometries in complexes of histones with DNA. It seems, therefore, that the nucleohistone with a lower histone/DNA ratio would be considered as a more specific complex if it assembles in conditions of a high excess of histones over DNA. With this in view it should be stressed that we obtain the nucleohistones with a protein/DNA ratio as low as 1.36-1.6 only after 16 h incubation of the histone/DNA mixtures. After a short incubation we could obtain the complexes with histone to DNA ratios above 3. This is in agreement with V o o r d o u w et al. [51], who supposed that histones as ligands with "multipoint attachment" are capable of forming non-specific precipitates in mixtures with DNA at near physiological ionic strength. However, this "open association" seems to be converted into a specific one during incubation of histone/DNA mixtures leading concurrently to the decrease in histone to DNA ratios of the complexes and to the selective exclusion of histone fractions with lower affinities for DNA from the resulting nucleohistones.

S t e i n et al. [45] noted that samples of DNA and core histones directly mixed in a medium of near physiological ionic strength (0.1-0.2 M NaCl) were initially quite turbid but cleared substantially during the incubation. According to biochemical and biophysical assays performed by S t e i n et al.[45] the nucleosomes can assemble in a medium of physiological ionic strength after 16 h incubation of DNA and histones directly mixed in the absence of any assembly factors. Slowly mixing DNA and total histone in media of near physiological ionic strength, R u i z-C a r r i l l o et al. [40] could essentially decrease the incubation period required for nucleosome reconstitution. Extract of Drosophila embryos (N e l s o n et al. [30]) and polyglutamic acid (S t e i n et al. [45]) were shown to stimulate the rate of nucleohistone assembly. However, this does not yet mean that the factors facilitating the chromatin assembly are necessary for cells, because the rate of nucleohistone reconstitution in vitro may be determined by the process of conversion of nonspecific aggregates into specific complexes.

H s i a n g & C o l e [13] have shown that a rapid mixing of DNA and histones at physiological ionic strength results in complexes much less reproducible and heterogeneous in shape than slow mixing does. In cells the mixing of chromatin components occurs by diffusion of protein molecules to DNA, i.e. without any shearing. Therefore, it cannot be excluded that so called "assembly factors" (L a s k e y et al. [24, 25]; R u i z-C a r r i l l o et al. [40]; N e l s o n et al. [30]; G e r m o n d et al. [10]; V a s h a k i d z e et al. [50]; M i l l s et al. [28]; S e n s h u & Y a m a d a [41]) are necessary only for nucleohistone assembly in vitro to prevent from the shear-induced formation of nonspecific aggregates or to increase the rate of disassembly of nonspecific complexes and to facilitate the transition of the system to thermodynamic equilibrium.

L a s k e y et al. [24, 25] and M i l l s et al. [28] suppose, however, that an acidic protein, purified from eggs of Xenopus laevis, organizes the histones into precursor complexes and transfers them to DNA to form nucleosomes. Unfortunately L a s k e y [25] and coworkers do not give any evidence in favour of histone transfer from one polyanion to another in the form of an octamer at physiological ionic strength. In contrast, it is known (R u i z-C a r r i l l o & J o r c a n o [39]) that under these conditions the free histone octamers dissociate into H3-H4

tetramers and H2A-H2B dimers. Moreover, we could show that such polyanion as heparin removes the histones H1, H2A and H2B, but not H3 and H4, from chromatin in 0.15 M NaCl (P a p o n o v et al. [34]). Thus the histone transfer from one polyanion to another seems to be accompanied by dissociation of nucleosome octamers into the complexes which are thermodynamically stable at physiological ionic strength. If a competitor polyanion is capable of dissociating all histone fractions, they can be assembled on it into the octamers. Indeed, S t e i n et al. [45] have shown that not only DNA or L a s k e y's factor but even polyglutamic acid is able to form the histone octamers in a medium of physiological ionic strength. In the absence of special control one can make a wrong conclusion about histone transfer from one polyanion to another in the form of octamers.

We know only one work (H i l d e b r a n d et al. [12]), where it was concluded that histones transfer from chromatin to a polyanion as octamers because the release of core histones from chromatin by increasing concentrations of heparin in medium of low ionic strength (0.01 M Tris-HCl, pH 7.4) was coordinate. However, these data contradict not only our experiments with heparin (P a p o n o v et al. [34]) but also results of I l y i n et al. [14], which have shown that increasing concentrations of such polyanions as DNA or tRNA remove the histones from chromatin at low ionic strength in the sequence: H1, H2A+ + H2B, H3 + H4. We cannot explain this contradiction but it is possible that apparent cooperative release of core histones from chromatin in work of H i l d e b r a n d et al. [12] was caused by decrease in differences of histone affinities for DNA in media of very low ionic strength that we shoed by studing a competitive histone binding with DNA (P a p o n o v et al. [34]).

If the histone octamers are disintegrated during transfer from one polyanion to another at physiological ionic strength (P a p o n o v et al. [35]) the role of chromatin assembly factors becomes uncertain. Therefore, we believe in accordance with other emthers (W o r c e l et al. [54]; R u i z-C a r r i l l o et al. [40]; S e n s h u & Y a m a d a [41]; M c G h e e et al. [27]) that chromatin assembly in vivo may occur by interaction of H3-H4 tetramers and H2A-H2B dimers on mascent DNA.

The major question in our studies was: can the histone competition for DNA take place in a cell?

Irrespective of the viewpoint on chromatin assembly our findings require the following data to be brought into mutual agreement.

1. The histone composition of nucleohistones, reconstituted both by direct mixing of total histone with DNA and by the salt gradient dialysis of their mixtures from 2M NaCl in physiological ionic strength, is dependent on input histone to DNA ratio, when it is greater than 1 (this paper). H1-free nucleohistones are formed at input histone/DNA ratio greater or equal to 1.5. In conditions of a high excess of the total histone over DNA the nucleohistone reconstituted contains only H3 and H4 histones(this paper and P a p o n o v et al. [34]).

2. The following histone/DNA ratios were reported for cell nuclei: 1.1 for calf thymus (C h a n d a et al. [6]); 2 for rat and calf livers (C h a n d a et al. [6]);3.3 for pea embryo axes (G r e l l e t et al. [11]) and HeLa cells (M u n r o et al. [29]); 20,000 for eggs of the frog Xenopus laevis (M i l l s et al. [28]).

3. Histone to DNA ratios for chromatin of the most of eukaryotic cells and in particular of rat liver and HeLa cells are 1.1 ±0.1 (F r e d e r i c q. [9]; K o r n b e r g [19]; B o n n e r & G a r r a r d [4]). We know only one work, where the histone to DNA ratio, as high as 3.3, was found for the chromatin of pea embryo axes (G r e l l e t et al. [11]) but this is in conflict with previous data which was 1.03 (B o n n e r et al. [3]).

4. Chromatin of the cells studied so far contains all five main fractions of histones.Core histones are present in chromatin preparations from various cells in approximately equimolar amounts (J o f f e et al. [15]; R a l l et al. [37]).

In the light of the data cited above we should conclude that the cell nuclei of calf thymus do not contain extrachromosomal histones, but the nuclei of rat liver, HeLa cells and eggs of Xenopus laevis contain various amounts of these histones. The histone to DNA ratios in the nuclei of the latter three species are so high, that histone H1 should be absent in their chromatins according to our results on nucleohistone self-assembly. However, no evidence was presented in favour of this.

It may be supposed that rat liver, pea embryos and HeLa cells contain a certain factor which prevents the histone competition

for DNA either by binding the additional complement of histones with chromatin (in pea embryos according to G r e l l e t et al. [11]), or by holding the histones out of chromatin in the state with equalized affinities of all fractions for DNA. This factor seems to be an acidic nonhistone protein assembling the histones into octamers or nonamers and containing only in the cells with a 2-fold or more weight excess of histones over DNA. Such histone excess was found in meiotic (M i l l s et al. [28]), embryonic (G r e l l e t et al. [11]) and polyploidic cells (M u n r o et al. [29]; C h a n d a et al. [6]).

When under certain conditions diploid cells synthesize histones in excess, the histone competition for DNA seems to occur because of the absence of the factor mentioned above. One of the examples illustrating this suggestion is the assembly of minichromosomes of SV40 in infected diploid cells. It is known that the histone to DNA ratio increases after SV40 infection (K a y & S i n g e r [17]). Recently L a B e l l a & V e s c o [21] have shown that histone H1 is associated with the chromatin of SV40 only at early stage of infection but is absent at later one. T a n [48] has shown in complete agreement with our finding in vitro that virions assembled in SV40-infected cells with 2-fold excess of total histone are free of H1 and depleted in H2A and H2B. No mechanism of this phenomenon was suggested by by the authors but it may be most simply explained by the histone competition for DNA in the absence of a "histone competition masking factor" (HCM factor).

It is possible that the chromatin assembly protein described by L a s k e y et al. [25] and by M i l l s et al. [28] is, in fact, the HCM factor. M i l l s et al. [28] do not also exclude the possibility that "the high concentration of this protein in the oocyte nucleus is an adaptation which permits the accumulation of a large histone pool".

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KOMPETYCYJNE WIĄZANIE HISTONÓW Z DNA I JEGO ZNACZENIE DLA STRUKTURY CHROMATYNY

S t r e s z c z e n i e

W pracy przedstawiono wyniki badań własnych i piśmiennictwa światowego nt. kompetycyjnego wiązania histonów z DNA. Zwrócono uwagę na fakt, że w przypadku mieszanin DNA z całkowitym histonem w warunkach fizjologicznej siły jonowej i przy stosunku wagowym histon/DNA > 1 tworzy się nukleohiston ze zmniejszoną zawartością histonu H-1. Natomiast przy stosunku histon/DNA $\geqslant$ 1,5 powstaje nukleohiston pozbawiony histonu H-1 i o zmniejszonej zawartości histonów H2A i H2B. Świadczy to o kompetycji histonów wiążących się z DNA, co może mieć istotny wpływ na właściwości strukturalne chromatyny zarówno in vitro, jak i in vivo.

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