NUCLEOSOME CORE PARTICLES CAN BE RECONSTITUTED USING MIXTURES OF HISTONES FROM TWO EUKARYOTIC KINGDOMS

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1. Introduction

It is now well known that nucleosome core particles can be reconstituted by mixing the four histones H2a, H2b, H3, H4 and the DNA under appropriate conditions (reviewed [1]). The most commonly used reconstitution procedure involves mixing of the histones and DNA in high salt (2 M NaCl) followed by a stepwise decrease of the ionic strength down to $\leqslant 0.25$ M NaCl. The nucleosome core particles reconstituted in this way have properties very similar to the native ones as judged by electron microscopy [2], sedimentation velocity [3], nuclease digestion [4] and the ability to impose a constraint to circular DNA [2].

There is much evidence that the histones H3, H4 and H2a, H2b play distinct roles in the assembly and stability of the nucleosome [2,5–8]. Histones H3, H4 have a central role in the formation of the nucleosome and are able to generate particles with properties similar to that of the nucleosome, whereas histones H2a, H2b are necessary to complete and stabilize the nucleosome. The most conserved histones are H3 and H4 and this may be related to their very precise role in chromatin structure. In contrast H2a and H2b have not been as well conserved during evolution. Moreover, variants of H2a and H2b appear sometimes during embryogenesis [9]. This suggests that H2a and H2b do not play a static structural role but that they may play different roles according to the functional requirement of the cell. It has also been suggested that the difference in nucleosomal DNA content observed for the various chromatins could be related to variations in the structure of H2a and H2b [2].

This prompted us to study the role of the different core histones on nucleosome structure by making reconstitution experiments with mixtures of histones from different species. Indeed, histones H2a and H2b from plants differ markedly in amino acid composition and size from their animal counterparts [10]. In a first approach we have compared the properties of the histone–DNA complexes obtained by mixing the DNA with histone H2a, H2b from one species (tobacco leaves or chicken erythrocytes) and H3, H4 from another species.

The reconstituted complexes have been characterized by 4 different criteria: DNA and histone content; sedimentation velocity; ability to impose a constraint to closed circular DNA; digestion by micrococcal nuclease and pancreatic nuclease DNase I. We find that nucleosome cores can be reconstituted with mixtures of tobacco H3, H4 and chicken H2a, H2b or vice versa and that they are indistinguishable. They are also similar to nucleosomes reconstituted with the 4 core histones from the same species.

2. Material and methods

Chicken erythrocyte histones and total tobacco leaf histones were isolated as in [11,12]. They were fractionated according to [13]. The histone pairs used in the reconstitution experiments were characterized by gel electrophoresis [14] and are shown in fig.1.

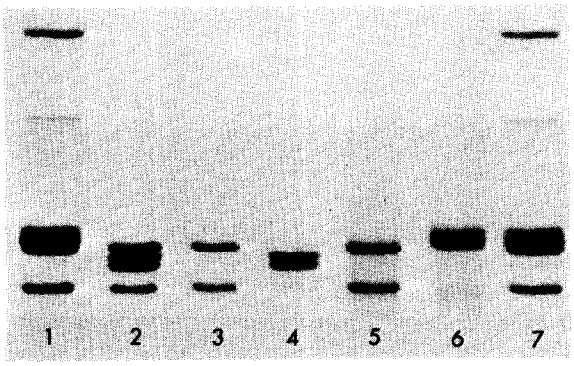

Fig.1. SDS–polyacrylamide gel electrophoresis of purified histones: (1,7) total histones of tobacco leaves; (2) chicken erythrocyte core histones; (3) chicken erythrocyte H3, H4; (4) chicken erythrocyte H2a, H2b; (5) tobacco leaf H3, H4; (5) tobacco leaf H2a, H2b.

The reconstitution experiments were made by dilution according to [15] or by gradient dialysis [3,5].

Nuclease digestion and DNA gel electrophoresis were performed as in [12].

Electron microscopy was performed according to [16]. The conditions for nicking and closing SV40 DNA and again gel electrophoresis were as in [15].

3. Results

3.1. *DNA content of the reconstituted core particles*

When SV40 DNA was reconstituted with various mixtures of histones, the resulting complexes showed a beaded appearance. Four different combinations of pairs of histones were examined: tobacco H3, H4 + tobacco H2a, H2b, tobacco H3, H4 + chicken H2a, H2b, chicken H3, H4 + chicken H2a, H2b and chicken H3, H4 + tobacco H2a, H2b. When the reconstitution was made at an histone/DNA ratio (w/w) of 1.2:1.5, 17–20 beads (~120 Å diam.) were associated with the SV40 DNA (fig.2A,B).

The yield of the reconstitution

$$= \frac{\text{observed no. nucleosomes}}{\text{maximum expected no. nucleosomes}} \times 100$$

for a given histone/DNA ratio was 80% and was the same for all combinations of histones.

The length of the DNA in the reconstituted beads was measured by electron microscopy. It was calculated by comparing the contour length of naked SV40 DNA and that of the reconstituted complexes. For all 4 combinations of pairs of histones it was found to be 188 basepairs ± 20. When 17–20 beads were associated with the SV40 DNA, the overall compactness of the DNA was about 2–2.3 in keeping with the results in [17].

3.2. *Histone content*

The histone content of the reconstituted core particles was analysed as follows: reconstitutes were obtained by mixing short DNA fragments (140–150 basepairs) extracted from purified core particles from chicken erythrocyte nuclei with different combinations of histones, at a histone/DNA ratio of 1. After centrifugation through a linear (5–25%) sucrose gradient 2 peaks were found and 70–80% of the DNA was present in a fast sedimenting peak which comigrates with native core particles used as control. The remaining material sediments more slowly and comigrates with free DNA. The material found in the fast sedimenting peak was analysed by electron microscopy and SDS gel electrophoresis. The classical beaded appearance of core particles was observed (fig.2C,D) in all cases.

The analysis of the histone content of the reconstituted core particles obtained with the various combinations of histones shows that the 4 core histones are present in the beads formed with all the mixtures of histones (fig.3). The histone stoichiometry cannot be deduced precisely from the gel electrophoresis data but very likely corresponds to equimolar amounts of each histone.

3.3. *Sedimentation velocity*

The nucleosome core particles reconstituted with short DNA comigrate with the native core particles on a sucrose gradient (see above) and have therefore sedimentation properties very similar to the native particles. The actual sedimentation coefficient was established by analytical ultracentrifugation. We have listed in table 1 the S values of the various reconstitutes, which are fairly similar to the values observed for native core particles.

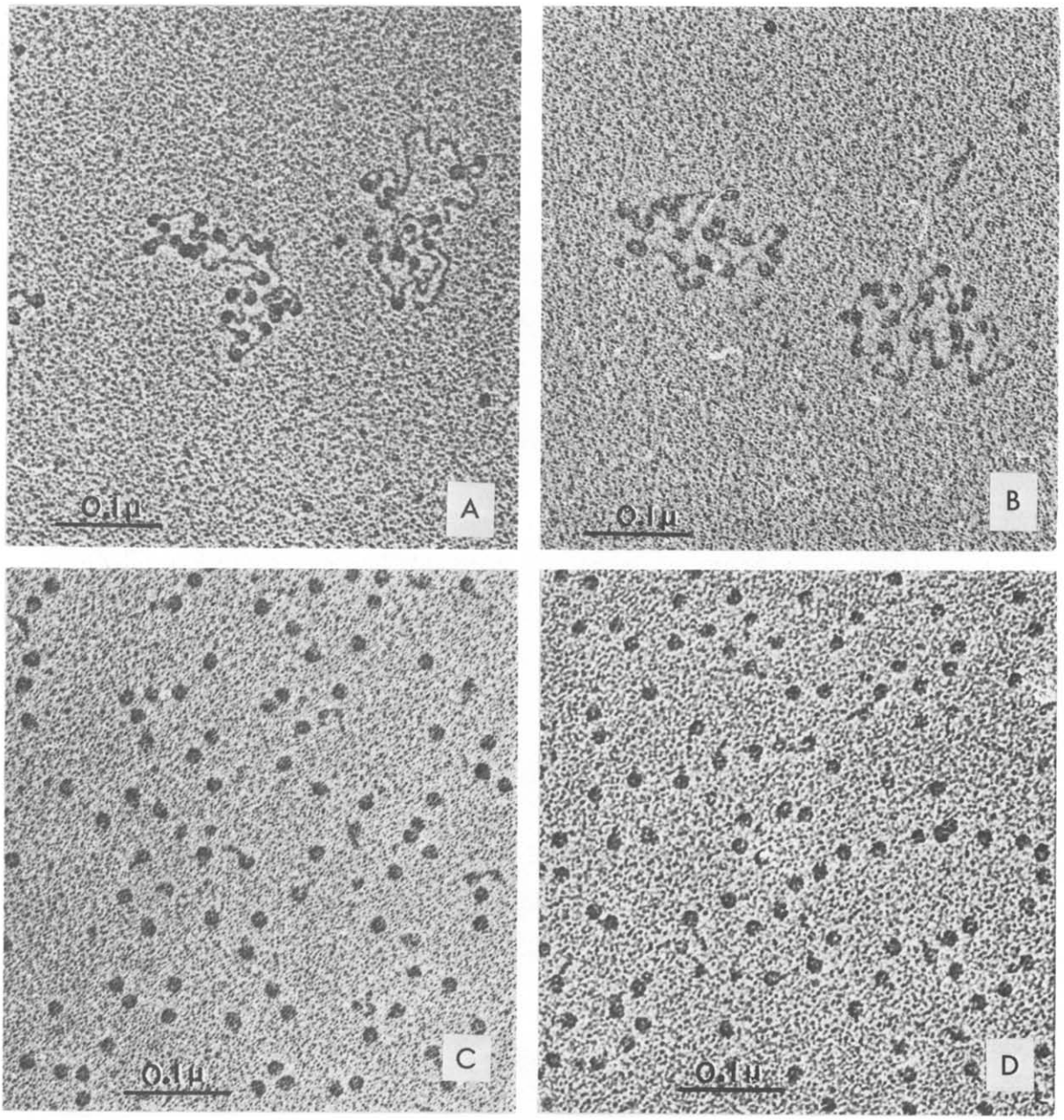

Fig.2.(A,B). Electron microscopy of SV40 DNA reconstituted with tobacco H3, H4 + chicken H2a, H2b (A) and chicken H3, H4 + tobacco H2a, H2b (B) (C,D) Electron microscopy of core particles reconstituted with tobacco H3, H4 + chicken H2a, H2b (C) or chicken H3, H4 + tobacco H2a, H2b (D).

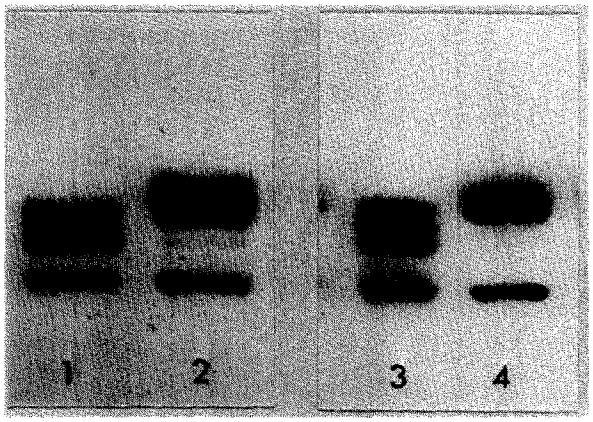

Fig.3. Histone content of the reconstituted core particles isolated by sucrose gradient centrifugation. The following mixtures of histones were used: (1) tobacco H3, H4 + chicken H2a, H2b; (2) Chicken H3, H4 + tobacco H2a, H2b; (3) chicken H3, H4 + chicken H2a, H2b; (4) tobacco H3, H4 + tobacco H2a, H2b.

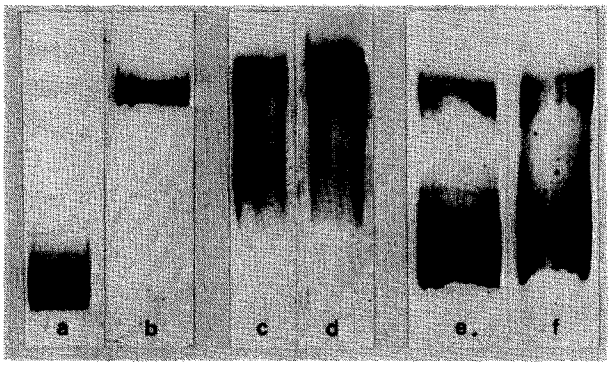

Fig.4. Gel electrophoresis analysis of SV40 DNA reconstituted with heterologous mixtures of histones and treated with the nicking–closing enzyme: (a) SV40 DNA I; (b) relaxed SV40 DNA I_r; (c,d) heterologous complexes at a histone/DNA ratio of 1 treated with the nicking–closing enzyme. Slot (c) corresponds to complexes with chicken H3, H4 + tobacco H2a, H2b and slot (d) to complexes with tobacco H3, H4 + chicken H2a, H2b (e,f) heterologous complexes at a histone/DNA ratio of 1.5. Slot (e) corresponds to complexes with chicken H3, H4 + tobacco H2a, H2b and slot (f) to complexes with tobacco H3, H4 + chicken H2a, H2b.

3.4. *Induction of superhelical turns*

Nucleosomes were shown [18] to impose a constraint to circular DNA. By comparing the gel electrophoresis and electron microscope data each nucleosome was shown to induce slightly more than one superhelical turn into SV40 DNA. We have used this method to examine the effect of the two heterologous combinations of histones on supercoiling.

Histones were reconstituted onto SV40 DNA I and, after the reconstitution, the complex was treated with the nicking–closing enzyme. The reconstitutes were then deproteinized and the DNA analysed by gel electrophoresis. The patterns obtained are shown in fig.4: slot (a) shows the pattern of the native SV40 DNA I and slot (b) the pattern of the relaxed SV40 DNA I after treatment of the naked DNA with the nicking–closing enzyme. When the two heterologous reconstitutes were treated with the enzyme, the pattern shown in slot (c) and (d) (histone/DNA ratio of 1) and (e) and (f) (histone/DNA ratio of 1.5) were obtained. When histones are bound to the DNA, the enzyme is unable to completely relax the SV40 DNA; the number of superhelical turns remaining after the treatment with the nicking–closing enzyme depends on the amount of histone bound to the DNA and accordingly on the number of beads associated with the SV40 DNA. In order to estimate the amount of supercoiling induced by one bead, the number of protected superhelical turns estimated from the gel electrophoresis pattern was compared to the number of beads seen in the electron microscope. We find that the beads formed with the heterologous mixtures of histones are able to protect the same number of superhelical turns (1–1.2) as nucleosomes reconstituted with the homologous mixtures.

Table 1
Sedimentation coefficient of reconstituted core particles

Histone mixture	Sedimentation coefficient ($s^0_{20,w}$)
Tobacco H3, H4–tobacco H2a, H2b	10.5
Tobacco H3, H4–chicken H2a, H2b	11.5
Chicken H3, H4–chicken H2a, H2b	10.6
Chicken H3, H4–tobacco H2a, H2b	10.6

3.5. *Nuclease digestion*

The extensive digestion of chromatin with staphylococcal nuclease gives rise to a series of discrete double-stranded DNA fragments. When reconstituted complexes of either homologous or heter-

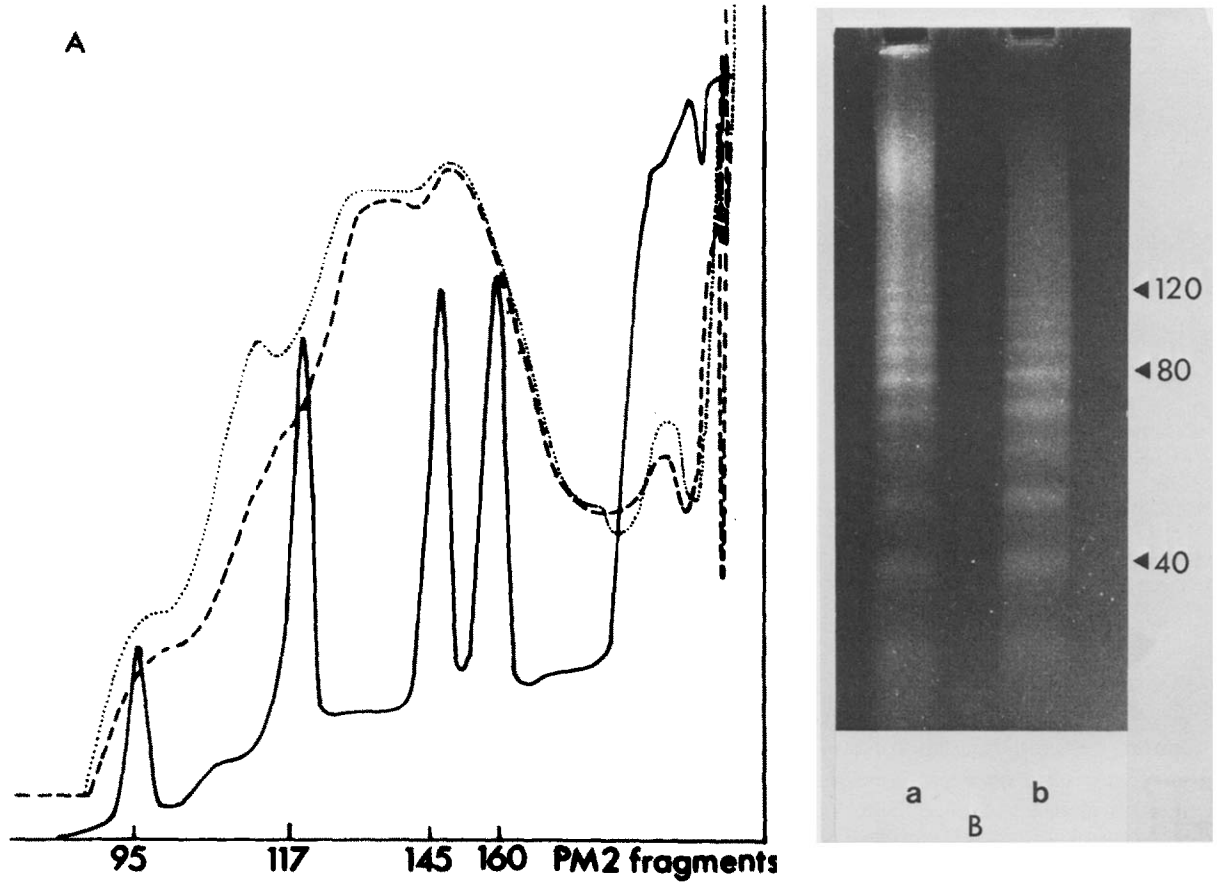

Fig.5. (A) Densitometer tracings of 6% polyacrylamide gels after extensive staphylococcal nuclease digestion of chicken erythrocyte DNA reconstituted with chicken H3, H4 + plant H2a, H2b (· · · ·) or tobacco H3, H4 + chicken H2a, H2b (- - - -). (B) DNA fragments resulting from a digestion with DNase I of chicken erythrocyte DNA reconstituted with (a) chicken H3, H4 + plant H2a, H2b and (b) plant H3, H4 + chicken H2a, H2b.

ologous mixtures of histones with sonicated chicken erythrocyte DNA were digested with staphylococcal nuclease, the limit digestion patterns showed several discrete bands (fig.5A) and were similar to the pattern obtained with native chromatin. The sizes of the limit digest bands are given in table 2. It has been shown by others [2,4] that extensive digestion of histone–DNA complexes containing only H3, H4 showed only fragments < 70 basepairs. On the other hand, the complexes lacking H3, H4 fail to give any discrete fragments. Since our reconstituted complexes give rise to the high molecular weight fragments we thus can conclude that they behave like true core particles. Evidence that the heterologous reconstitutes are identical to native particles also comes from digestion with pancreatic nuclease DNase I. When the DNA extracted from heterologous reconstitutes after

Table 2
Length of the DNA fragments after extensive digestion by the staphylococcal nuclease (see fig.5)

Fragment no.	Native chromatin (basepairs)	Heterologous reconstitutes (basepairs)
1	145	148
2	123–130	128
3	109	108
4	93	96
5	73	–

DNase I digestion is analysed on polyacrylamide gels under denaturing conditions (fig.5B), we observe indeed the characteristic band pattern indicating single strand cuts every 10 bases. The entire series of fragments is produced and the relative rates of cleavage (notice particularly the higher intensity of the band at 80 bases) is nearly identical with those obtained with native chromatin.

4. Conclusion

This paper addresses the question whether nucleosomes or nucleosome cores can be formed by reconstituting mixtures of histones from different species onto DNA.

If the nucleosome core is defined as a particle containing 2 each of the 4 histones H2a, H2b, H3 and H4 associated with 140 basepairs of DNA our results show indeed that core particles are formed with heterologous mixtures of histones despite the difference in size and amino acid composition between the slightly lysine rich histones H2a, H2b of the 2 species. The same kind of interaction must therefore occur between the histones in the interspecies complexes and in the intraspecies complexes. The pairwise interaction of histones, one each from two different species (pea and calf) has been similarly studied [19] and interkingdom complexes shown to be similar to those formed by histones of the same species as judged by the K_a between the different histones. This result is in agreement with our result that nucleosomes formed with an interkingdom mixture of histones are similar to nucleosomes formed with the 4 histones of the same species.

When the DNA content of the beads formed onto SV40 DNA is measured we find that the core histones are able to organize $>$ 140 basepairs in the heterologous complexes as well as in the homologous complexes. A length closer to that of the chromatin DNA repeat is obtained but however different from that of chicken or tobacco native chromatin. This result is in agreement with the conclusion [20] that H2a and H2b are unable to measure out specifically the linker DNA in reconstituted chromatin.

In conclusion, our results show that the histone complex formed by H3 and H4 can be interchanged between two species. It would be interesting now to investigate whether all the tobacco and chicken histones can be exchanged individually especially in view of result [21] where histone H4 of *Tetrahymena* was shown not to substitute for calf thymus H4.

Experiments are also in progress to study the competition between the histones of the two species and to see whether or not octamer containing homologous histones are preferentially made when the 2 sets of histones are allowed to compete.

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