HETEROGENEITY OF NUCLEOSOMES IN GENETICALLY INACTIVE CELLS

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1. Introduction

During the last few years reports have indicated that the core particles of nucleosomes, which are complexes of DNA ~140 basepairs in length with a histone octamer (H4 + H3 + H2A + H2B) × 2 (reviews [1,2]) are heterogeneous in regard to their protein content. Strong evidence for the heterogeneity of the histone octamer was the discovery of the histone variants [3,4]. Analysis of histone composition in the nucleosomes of somatic cells performed using two-dimensional electrophoresis has shown that all the histone variants discovered previously are present in the nucleosomes [5]. The functional meaning of nucleosome heterogeneity is currently discussed in connection with chromatin structural modulations which are thought to be involved in differential gene activity [1,2,5].

As is well known, the genome of sperm cell is completely switched off. The chromatin of sea urchin sperm has been shown to have the typical nucleosomal organization [6–8]. Here we report that in sea urchin sperm chromatin the core particles of the nucleosomes are heterogeneous in respect to their protein composition, which indicates that structural differentiation occurs in chromatin even when gene activity is completely suppressed.

2. Materials and methods

Sperm cells of the sea urchins, *Strongylocentrotus intermedius* and *Scaphechinus mirabilis* were used. Collection of sperm, isolation of nuclei, and total histone extraction have been described [9]. Digestion of the nuclei with micrococcal nuclease and preparation of the nucleosomes were as in [8]. Briefly the nuclei at 20 A_{260}/ml were digested with micrococcal nuclease (Worthington, USA) at 30 units/mg DNA for 15 min at 37°C.

The reaction mixture was chilled on ice to stop the digestion. The samples were centrifuged at 6000 rev./min for 2 min and the supernatant was discarded. The nuclear pellet was lysed in buffer containing 5 mM Tris–HCl, 2 mM EDTA, 0.1 mM phenylmethylsulfonylfluoride (pH 7.5). After 20 min the samples were centrifuged for 10 min at 6000 rev./min. The supernatant contained the soluble chromatin fragments.

The chromatin fragments were separated on 5% polyacrylamide gels in 10 mM Tris–borate (pH 8.3) containing 1 mM EDTA as in [10]. Electrophoresis in the second dimension was done as in [8]. Electrophoresis of the histones was according to [11].

3. Results and discussion

Histone H2B from sea urchin sperm differs significantly from its counterparts from other sources and is represented by 1–3 subfractions according to the species from which the protein originates [9,12,13].

Fig.1 shows electrophoretograms of total histone from the sperm of *Str. intermedius* and *Sc. mirabilis* in which histone H2B consists of 2 and 3 electrophoretic subfractions, respectively.

It is important to know whether all the variants of histone H2B are present in the nucleosomes of the genetically inactive chromatin of sperm cells. To answer this question the protein composition of the nucleosomes was determined using two-dimensional electrophoresis.

Chromatin fragments resulting from digestion of nuclei with micrococcal nuclease were separated on 5% polyacrylamide gel (fig.2,I). The bands corre-

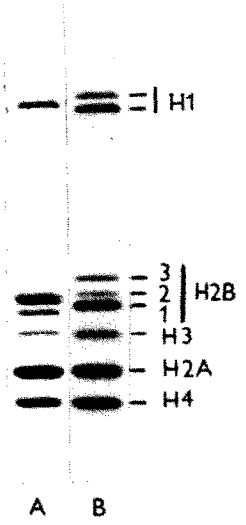

Fig.1. Polyacrylamide gel electrophoresis of histones from sperm of the sea urchins: (A) *Strongylocentrotus intermedius;* (B) *Scaphechinus mirabilis.* Electrophoresis was according to [11].

sponding to the core particles (MN–H1), to the complete mononucleosomes (MN + H1), to the dinucleosomes (DN) and so on are seen on the electrophoregrams in the first dimension.

Second dimension electrophoresis shows that all the variants of the histone H2B are contained in each type of chromatin fragment, that is in oligo-, tri-, di- and mononucleosomes including the core particles (fig.2,II).

A nucleosome core contains 2 molecules of each 'small' histone – (H4 + H3 + H2A + H2B) × 2. In accordance with these results, 2 types of histone H2B combination within a nucleosome are possible: presence in the same nucleosome of 2 identical or 2 different H2B molecules. In the first case the number of nucleosomal types differing in histone H2B will be equal to the number of H2B subfractions, that is 2 or 3 for the sea urchin species studied here. In the alternative case when a nucleosome contains different subfractions of histone H2B, 3 combinations may exist in chromatin from sperm of *Sc. mirabilis* [(H2B1,

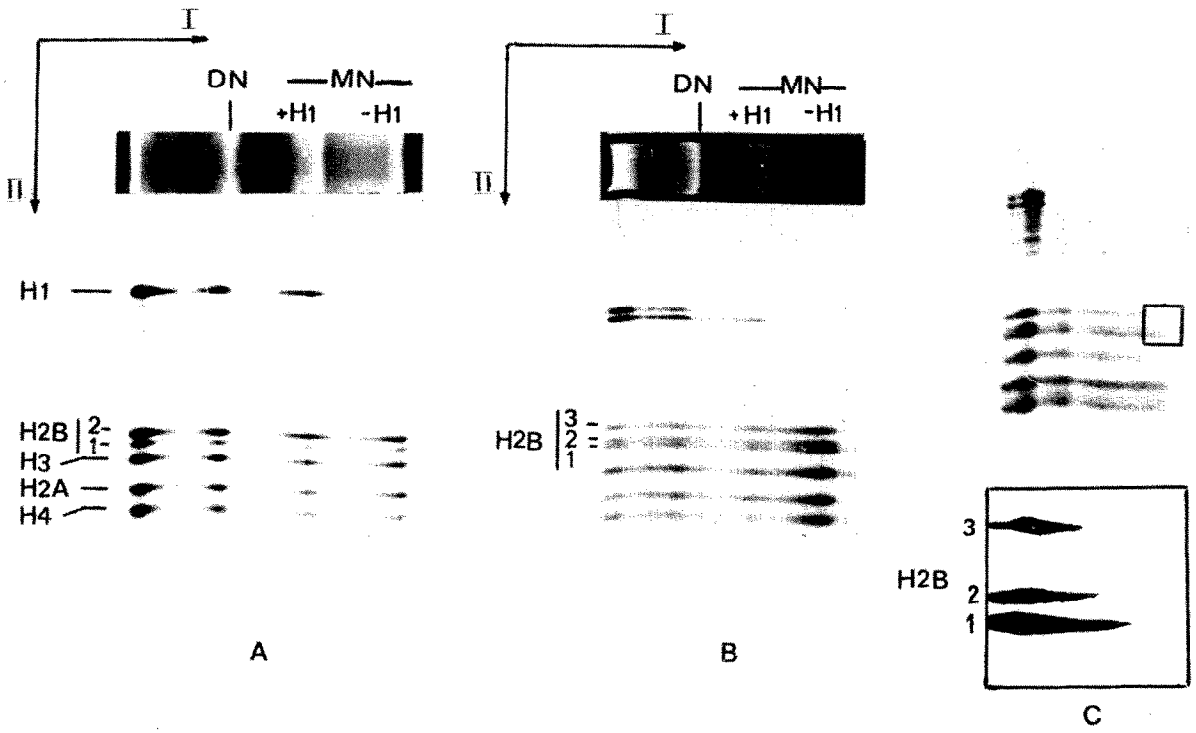

Fig.2. Two-dimensional electrophoresis of the nucleosomes from the sperm of the sea urchins: (A) *Strongylocentrotus intermedius;* (B,C) *Scaphechinus mirabilis.* (I) First dimension; low-ionic-strength electrophoresis of the chromatin fragments on 5% polyacrylamide gel; (II) second dimension, separation of the proteins contained in the nucleosomes on SDS–polyacrylamide gel [11]; DN, dinucleosomes; MN, mononucleosomes. (C) Photograph and enlarged schematic representation of region of the gel included in the square to show the shift of H2B subfractions.

H2B2), (H2B1, H2B3) and (H2B2, H2B3)] and one combination (H2B1, H2B2) in sperm chromatin of *Str. intermedius*. At the same time in the latter along with nucleosomes containing different H2B molecules, nucleosomes with identical H2B subfractions, present in redundancy, should occur in sperm chromatin.

The nucleosomes containing various combinations of histone H2B subfractions differ too little to be well resolved on polyacrylamide gel. However, in the second dimension electrophoresis of nucleosomal proteins a characteristic shift of H2B subfractions could be noticed (fig.2B,C). Data of fig.2C show that the material corresponding to the chromatin fragments with the highest mobility in first dimension electrophoresis contains only one H2B subfraction, H2B1 (lowest M_r). This indicates that particles with identical H2B subfractions may exist.

The essential conclusion from these findings is that the population of sperm nucleosomes in both sea urchin species is obviously heterogeneous. Variable in the H2B subfractions are their N-terminal parts as has been shown by primary sequence analysis [12,13]. These regions are significantly elongated as compared with H2Bs from other sources and represent entirely new structural domains which contain variable numbers of repeated basic pentapeptides Pro–Thr–Lys–Arg–Ser or Pro–Arg–Lys–Gly–Ser. Such domains can be involved in DNA condensation [13].

The character of nucleosome heterogeneity in sea urchin sperm chromatin is certainly different from that of somatic cells. In the latter, histone variants differ by 1–3 amino acid subfractions in hydrophobic parts of the molecules which are presumably responsible for histone–histone interactions in the protein core of a nucleosome. In sperm cells variation is connected with the N-terminal part of histone H2B, which is thought to interact with DNA.

Earlier we have obtained data indicating that in sea urchin sperm chromatin the N-terminal region of histone H2B can be bound to the linker DNA and, like histone H1, is involved in the formation of the higher order structure of sperm chromatin [8]. In this sense the heterogeneity of nucleosomes in respect to histone H2B may result in modulation of the chromatin structure at supranucleosomal level.

The biological meaning of such modulations in genetically inactive cells is unclear. It is known that the sperm-specific histones in sea urchins are completely replaced after fertilization by embryonic ones [14]. In this connection the variation in nucleosomal structure or modulations in supranucleosomal organization may carry epigenetic information necessary to provide the correct distribution of embryonic histones and/or other chromosomal proteins on the paternal DNA.

References

[1] Chambon, P. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 1211–1236.
[2] Tsanev, R. (1978) in: The Cell Nucleus' (Busch, H. ed) vol. 4, pp. 107–133, Academic Press, New York.
[3] Franklin, S. G. and Zweidler, A. (1977) Nature 266, 273–275.
[4] Cohen, L. H., Newrock, K. M. and Zweidler, A. (1975) Science 190, 994–997.
[5] Baffus, N. L., Albright, S. C., Todd, R. D. and Garrard, W. T. (1978) J. Biol. Chem. 253, 2658–2674.
[6] Spadafora, C., Bellard, M., Compton, I. L. and Chambon, P, (1976) FEBS Lett. 69, 281–285.
[7] Keichline, L. D. and Wassarman, P. M. (1979) Biochemistry 18, 214–219.
[8] Zalenskaya, I. A., Pospelov, V. A., Zalensky, A. O. and Vorob'ev, V. I. (1981) Nucleic Acids Res. 9/3, in press.
[9] Zalenskaya, I. A. and Zalensky A. O. (1980) Comp. Biochem. Physiol. 65B, 369–373.
[10] Pospelov, V. A., Svetlikova, S. B., and Vorob'ev, V. I. (1977) Nucleic Acids Res. 4, 3267–3279.
[11] Laemmli, U. K. (1970) Nature 227, 680–685.
[12] Strickland, M., Strickland, W. N., Brandt, W. F. and von Holt, C. (1977) Eur. J. Biochem. 77, 263–275.
[13] Von Holt, C., Strickland, W. N., Brandt, W. F. and Strickland, M. S. (1979) FEBS Lett. 100, 201–217.
[14] Poccia, D., Salik, J. and Krystal, G. (1978) J. Cell Biol. 79, 121.