HISTONE-CONTAINING SUBNUCLEOSOMES: POSSIBLE IMPLICATIONS TO NUCLEOSOME STRUCTURE

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1. Introduction

Earlier work in Felsenfeld's laboratory showed that extensive digestion of chromatin with micrococcal nuclease results in a limit digestion set of discrete double-stranded DNA fragments of sizes ranging from about 130 base pairs (bp) to 45 bp [1]. Alternatively, mild treatment of chromatin with nucleases produces a soluble mixture of mononucleosomes and their oligomers which can be separated from each other by sucrose gradient centrifugation [2,3] or by polyacrylamide gel electrophoresis [4–6]. However, polyacrylamide gel electrophoresis of DNA · protein under non-denaturing conditions has proven to be a more successful method in studying the heterogeneity of nucleosomes and their composition. We have separated 4–5 main structural types of mononucleosomes: along with core particles (MN1), mononucleosomes were identified containing an additional segment of linker DNA and either H1 histone or non-histone protein HMG 14 or 17 [6–8]. We also discovered a set of small subnucleosomal particles which contained short DNA fragments and histones or non-histone proteins [3,6,7]. Two of them (SN2 and 3) comprising short DNA fragment and HMG protein originated apparently from transcriptionally active chromatin [8].

Here, we present the composition of two major histone-containing subnucleosomes, which can be solubilized from chromatin by different DNases [8]. We were able to reveal their origin from mononucleosomes and oligomers. This precursor–product relationship suggests some considerations which may be relevant to the nucleosome structure.

2. Experimental

Techniques for both cell cultivation and the isolation of nuclei from mouse L cells have been published in [8]. The isolated nuclei were suspended in buffer A [0.04 M NaCl, 2 mM $MgCl_2$, 10 mM triethanolamine (TEA)–HCl, pH 7.6] containing 1 mM $CaCl_2$ at 5×10^8 nuclei/ml. Then micrococcal nuclease (Worthington) was added to 75 units/ml and the digestion carried out at 37°C. The reaction was terminated by chilling in ice and the first supernatant (S1) was obtained by centrifugation at $5000 \times g$. The digested nuclei were washed with buffer A and lyzed by addition of 2 mM Na-EDTA (pH 7.6). The second supernatant (S2) constituted of mono- and oligonucleosomes was prepared. Both the supernatants were dialyzed against 1 mM Na-EDTA (pH 7.6) and clarified by centrifugation.

In order to trace the precursor–product relationships between mono- and subnucleosomes, the electrophoretically separated nucleosomal subfractions [5,7] were digested directly in a polyacrylamide gel matrix as in [9] with a primary concentration of 75 units nuclease/ml. Then the DNA · protein products of digestion were recovered using an electrophoresis in the second direction. Alternatively, mononucleosomes were labeled at their 5′-termini using polynucleotide kinase and [γ-^{32}P]ATP (Radiochemical Centre, Amersham) [10]. Labeled mononucleosomes were also subjected to 'chromatin fingerprinting' [9].

Analysis of protein composition of mono- and subnucleosomes was done as in [7].

3. Results

The treatment of purified chromatin with micrococcal nuclease or with DNase I or II produces a set of subnucleosomes, in particular histone containing SN4 and 7 [6–8]. However, it is possible that these

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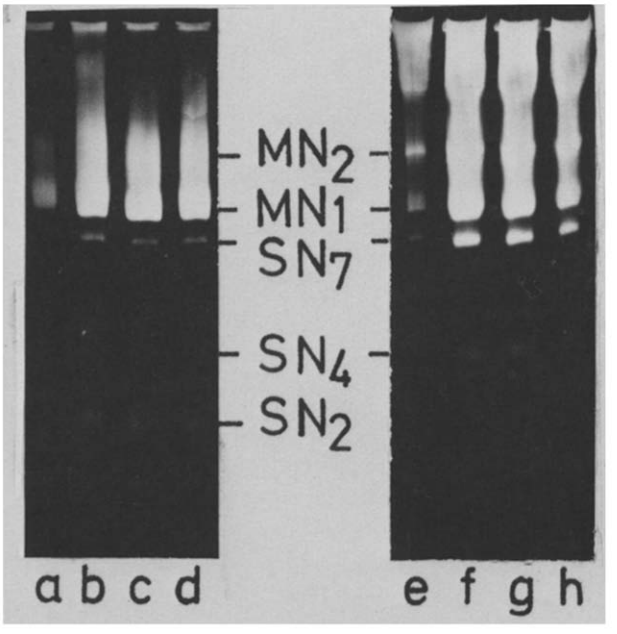

Fig.1. Polyacrylamide gel fractionation of DNA · protein produced by nuclease treatment of nuclei. Purified nuclei were digested to 3%, 8%, 18% and 28% of acid-soluble DNA and S1 (a–d) and S2 (e–h) were applied to a 6% gel.

particles originate from some fraction of chromatin whose supranucleosomal and nucleosomal organization were disturbed during chromatin isolation. Fig.1 rules out this possibility and demonstrates that SN4 and 7 are released during digestion of nuclei. The both types of particles are present in S1 and S2. A detailed analysis of the protein content and the lengths of DNA fragments in subnucleosomes is able to indicate the type of structures from which they are produced.

3.1. *The composition of subnucleosomes SN4 and 7*

We have therefore obtained SN4 and 7 by sucrose gradient fractionation followed by additional electrophoretic purification [6]. We had roughly estimated the length of DNA fragments from these particles, and found that SN4 contained fragment about 40 bp long. A precise measuring the length of single-stranded DNA fragments from SN7 with the aid of Noll's DNase-I-produced ladder [10,11] indicates that they have a size from 106–110 bp (not shown).

SN7 particles were shown to contain all 4 core histones. A direct two-dimensional analysis of the protein composition of SN7 reveals an ~2-fold excess of histones H3 and H4 as compared to H2a and H2b (fig.2). For comparison, the densitogram of histones

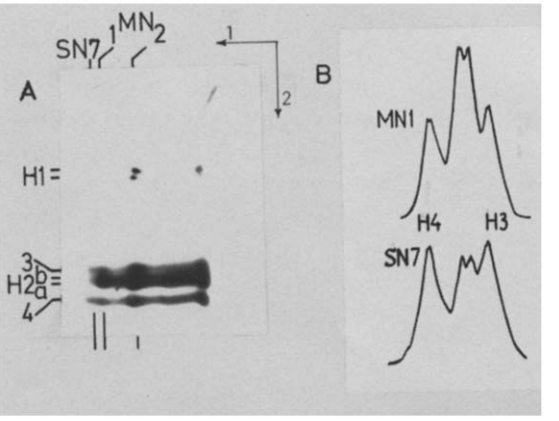

Fig.2. Analysis of histone composition of subnucleosome SN7. [^{14}C]Protein-labeled nucleosomes were separated in 6% polyacrylamide gel. This gel was placed above 15% dodecylsulfate slab gel and subjected to electrophoresis in the second direction to display protein. Then the gel was impregnated with PPO and exposed to a Kodak film XR-2 (A). Histone fractions of SN7 and MN1 were scanned along indicated vertical lines at 650 nm on an Opton densitometer (B).

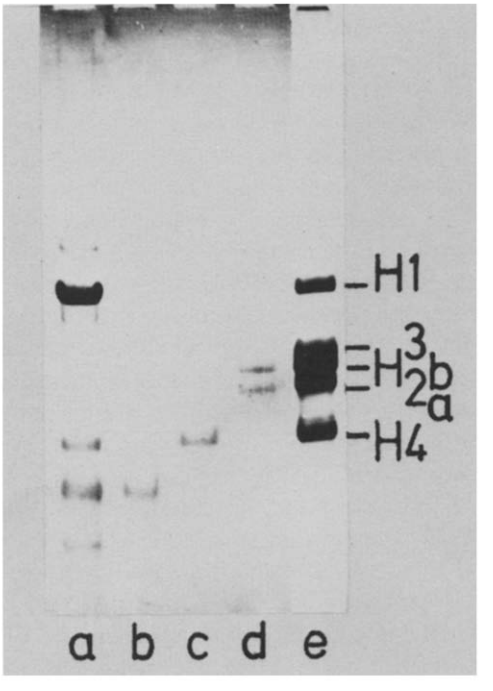

Fig.3. Protein composition of subnucleosome SN4. Proteins from subnucleosomes SN2, SN3, SN4 and chromatin were extracted with 0.4 M H_2SO_4 and dialyzed against 5% PCA. Soluble and insoluble material was concentrated with ethanol and analyzed by polyacrylamide gel electrophoresis in acetic acid–urea medium: (a) $HClO_4$-soluble fraction of chromatin; (b,c) soluble fraction of SN2 and SN3; (d) insoluble fraction of SN4; (e) total histones.

from core particles (MN1) was used where all the core histones were present in equimolar amounts. (There are differences in the incorporation of the ^{14}C-label into different histone fractions. ^{14}C-labelled amino acids were incorporated into H2a and H2b at a higher rate than into H3 and H4 in this particular experiment.) So, one may suppose the presence of a tetramer $(H3–H4)_2$ plus a dimer (H2a–H2b) for SN7. In comparison with core nucleosomes, subnucleosomes SN7 lack one pair of H2a + H2b.

Although we have reported about the proteins of SN4 [6,7] the final composition remained obscure. The proteins of SN4 comigrated with histones H2a and H2b both in dodecylsulfate polyacrylamide gel (see fig.4 in [7]) and in acetic acid–urea gel (fig.7 in [7]). However these proteins have a mobility which was close to that of some HMG proteins or their degradation products. To select between the two possibilities, we purified these proteins and compared their solubility in 5% perchloric acid with that of HMG proteins [12] which were present in subnucleosomes SN2 and 3 (fig.3). It can be seen that incubation with PCA resulted in precipitation of SN4 proteins though proteins from SN2 and 3 remained in solution. Consequently, SN4 contains histones H2a + 2b rather than HMG proteins.

3.2. *Subnucleosomes SN4 and 7 originate from mononucleosomes MN1*

As a direct approach to elucidating a precursor of SN4 and 7, the 'chromatin fingerprinting' method [9] has been used. This technique allows one to obtain a particular set of particles originating from nucleosomes (see fig.4). SN7 is the most discrete product of such a nuclease degradation. The appearance of SN7 is also evident by 'fingerprinting' of mononucleosome preparation labeled in vitro at 5′-ends with the aid of [γ-^{32}P]ATP and polynucleotide kinase. In this case, we also observe a visible spot with a mobility close to that of SN4. However, H1-containing MN2 are relatively resistant to digestion of terminal label as shown in [13].

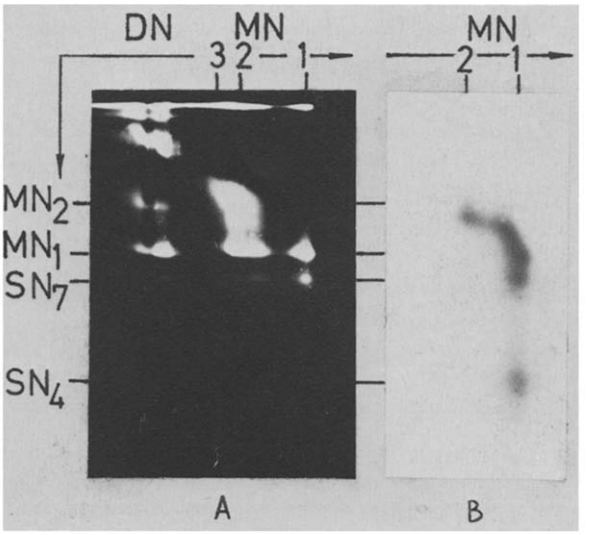

Fig.4. Mapping precursor–product relationship by nuclease treatment of nucleosomes: (A) ethidium bromide staining; (B) in vitro labeled mononucleosomes were processed and gel was exposed to a Kodak film.

4. Discussion

Subnucleosomal particles that may be related to SN7 and 4 have been observed in [5,14]. However, the precise composition of these particles and their origin remained unclear. Our data on the composition of SN7 give a figure of 6 histone molecules (H2a, H2b, 2H4, 2H3)/~108 bp of DNA. The second studied subnucleosome SN4 consists of ~40 bp DNA fragment complexed with H2a and H2b that are present in about equal amount (see fig.3). In view of the suggestion that one molecule of H2a or H2b protects 15–20 bp of DNA [15], it seems likely that only one pair of these histones is present in SN4. It must be noted that the sum of the lengths of SN4 and 7 DNA fragments is close to the size of a core DNA. Moreover, histone sets of these subnucleosomes form a complete octamer of the core.

The most interesting result of this study is the nuclease splitting of core nucleosomes; as a result, a pair of complementary subnucleosomes SN7 + SN4 is formed. These data indicate a special location of the H2a + H2b pair in the core; it might be localized near at least one end of the 145 bp core DNA and could be released by nuclease in a complex with the 40 bp DNA fragment. This conclusion is confirmed by nuclease cleavage of 5′-ends labeled core (MN1) which preserve the terminal label at one of the ends of the both SN7 and SN4 particles (see fig.4b).

The abundant cross-linking of H2a to H1 both zero-length and more distant reagents [16,17] suggests the terminal localization of H2a in the nucleosomal core. These data and our results enable us to

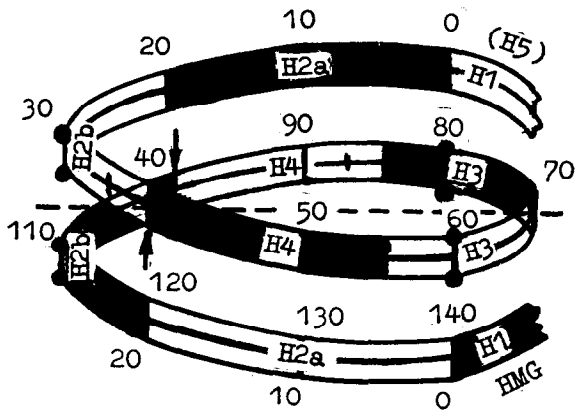

Fig.5. A model of H1-containing mononucleosome. Histones are shown as shaded and open regions extended along the DNA (without detailed localization of their molecules). The arrows indicate the points of nuclease attack resulting in the formation of subnucleosomes SN7 and SN4.

orient the H2a–H2b–H4 histone oligomer chain (discovered in [18]) along DNA in a nucleosomal core (see fig.5). In the model we used the left-handed superhelical coil of 140–145 bp DNA proposed [19] as a basis for a linear histone array [20]. The DNA coil was extended by ~10 bp at each end [13] to obtain 165 bp H1-containing MN2 [6]. This additional segments can interact with H1 (H5) histone or with non-histone proteins, especially HMG type [6,7,21]. The model being not so detailed as that in [15] reflects only the major sites of histone contacts with DNA. Eight histones of the core are arranged in the following sequence: H2a–H2b–H4–H3–H3–H4–H2b–H2a. Histones H3 and H4 are capable of forming a central kernel [22]. The histone chain proposed here is close to the array obtained with the use of another approach (cross-linking of histones to nucleosomal DNA) [15,23]. This model permits us to account for a number of different experimental findings bearing on histone–histone interactions. All histones which crosslinked to a higher extent are positioned in close proximity (H1–H2a, H1–H3, H2a–H2b, H2b–H4, H4–H3, H3–H3). Alternatively, the distant location of H4 histones from one another confirms the absence of this dimer in many experiments [17,24]. Finally, one might speculate that H2a–H2b containing terminal regions may unfold yielding a more accessible structure. It remains to be determined whether such unfolding does take place in vivo and, if so, what its biological significance is.

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