LEVELS OF GRANULAR ORGANIZATION OF CHROMATIN FIBRES

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1. Introduction

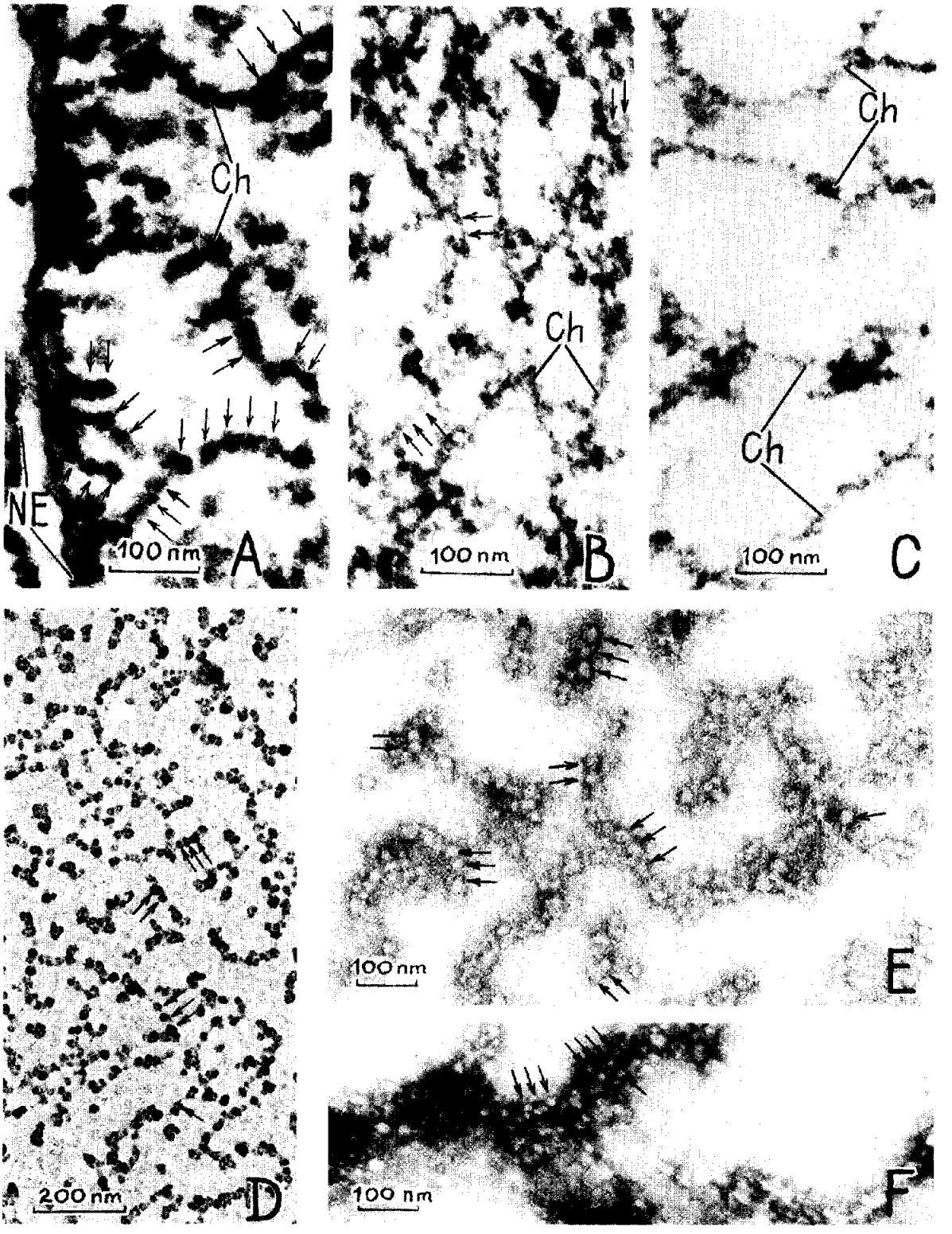
The molecular organization of chromatin is a much discussed problem. One of the questions is the size of fibrils in isolated deoxyribonucleoprotein (DNP) and its relation to the size of fibrils in intact nuclei. Recently a number of authors have reported the detection of spherical chromatin untis 80–100 Å in diameter connected to each other by a DNA strand [1–4]. However, according to many morphological descriptions chromatin fibrils have an average diameter of 200–250 Å [5–7]. It is also known that the diameter of chromatin fibrils in nuclei and DNP preparations may vary from 100 to 200 Å, depending on the isolation procedure used [8]. The aim of this study was to elucidate the factors determining the levels of structural organization of chromatin fibrils and to understand the way in which globular components participate in the formation of the 200 Å chromatin fibril. We have shown that the basic structural unit of chromatin is a fibril consisting of 200 Å globules which converts into a fibril with 100 Å globules when part of the magnesium is removed.

2. Materials and methods

For all experiments nuclei were isolated from rat liver, homogenized in a 30 mM sodium phosphate, pH 6.2, 3 mM Mg^{2+} and 0.32 M sucrose solution, purified by centrifuging through the homogenization solution with 2.3 M sucrose and resedimented in the homogenization solution. For autodigestion by endogenous nuclease, the nuclei were incubated according to [4] at 37°C for 60–90 min in 30 mM sodium phosphate, pH 6.2, 10 mM Mg^{2+} and 1 mM Ca^{2+} solution; then the nuclei were transferred into a solution containing 20 mM triethanolamine (TEA) and 1 mM Mg^{2+} and disintegrated in an MSE ultrasonic disintegrator. Nucleoli, dense chromatin and intact nuclei were removed from the nuclear homogenate and a globular chromatin fraction was then sedimented by centrifugation at 100 000 *g* for 60 min. The DNA and RNA content of the preparations was determined by Spirin's method [9], and the protein content according to Lowry [10]. Histones were extracted with 0.25 M HCl and analyzed electrophoretically in polyacrylamide gel by the method of Panyim and Chalkley [11]. For electron microscopy the samples were fixed by adding up to 0.25% neutralized glutaraldehyde to the suspensions containing the isolated fractions, and the embedded in Epon. The same samples were studied in an electron microscope after negative staining (2% uranyl acetate in 70° ethanol) or after shadowing with palladium.

3. Results

The average diameter of rat liver DNP fibrils in situ, as seen in ultrathin sections, is about 200 Å. The fibrils have the same diameter when the isolated nuclei are transferred into a solution containing 20 mM TEA and 1 mM Mg^{2+}. In these conditions chromatin is dispersed, which allows one to observe a sizeable part of the structure of a separate DNP fibril. At a high magnification (fig.1A) it is seen that each fibril consist of a row of globules, lying close together. The globules having an average diameter of 200–250 Å. In a solution containing 20 mM TEA without magnesium the fibers in the nuclei have an average diameter of 100 Å. These fibrils also


Ch
NE
100 nm
A
Ch
Ch
100 nm
B
Ch
Ch
100 nm
C
200 nm
D
100 nm
E
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F

Table 1
Chemical composition of chromatin and fractions of granular chromatin

Source of chromatin	mg of histone/mg of DNA	mg of non-histone protein/mg of DNA	mg of RNA/ mg of DNA
Nuclei extracted with 20 mM TEA and 1 mM Mg^{2+}	1.22	1.02	0.1
Nuclei extracted with 20 mM TEA without Mg^{2+}	1.15	1.05	0.09
Nuclei extracted with 20 mM TEA and 6 mM EDTA	1.20	0.96	0.08
Granular chromatin	1.20		0.08

contain globules, but of smaller diameter – about 100 Å (fig.1B). This structural transition is to a certain extent reversible. On addition of Mg^{2+} to 1 mM the thickness of the fibrils is restored to 200 Å, but the bulk of the chromatin fibrils do not retain their globular character. A second structural transition is observed after further removal of Mg^{2+} by EDTA (6 mM EDTA to 1 mM Mg^{2+}), the diameter of chromatin fibrils then decreases to 50–70 Å and they entirely lose their globular structure (fig.1C). Chemical analysis shows the DNA to histone ratio to remain constant in all these conditions, whereas the DNA to non-histone protein ratio ranges from 1 to 0.8 (see Table 1). Extraction of the major part of the non-histone protein from the nucleus in the presence of Mg^{2+} does not cause structural transitions of the fibril. Therefore these transitions are Mg-dependent, and a 200 Å fibril may be regarded as the basic chromatin structural unit. We tried to demonstrate the subunit nature of DNP fibrils by treating the nuclei with endogenous Ca-Mg-dependent nuclease. The nuclei were incubated in magnesium-containing solutions, in which DNP fibrils retain the 200 Å diameter.

A fraction of globular chromatin isolated in 20 mM TEA and 1 mM Mg^{2+} (see Materials and methods) is a heterogenous preparation composed of individual 200 Å globules and of chains of various length consisting of stacked globules of the same diameter (fig.1D,E). Both in the preparation of isolated nuclei and in the globular chromatin fraction, partial removal of Mg^{2+} brings about a structural transition in chromatin. Fig.1F demonstrates that an EDTA-treated fraction of globular chromatin is a chain of globules of about 100 Å diameter located at a distance of 300–400 Å from each other on filaments of 20–40 Å diameter. Chemical analysis of the globular chromatin fraction showed that the histone/DNA/RNA ratio did not differ from that for total chromatin and was 1.2 : 1 : 0.08 (see Table 1). All histones are present in this fraction, including the HI histone fraction (see fig.2).

Fig.1. Structure of chromatin fibrils in isolated nuclei (A,B,C) and in the globular chromatin fraction isolated in 20 mM TEA and 1 mM Mg^{2+} solution (D,E,F). (A) Nuclei in 20 mM TEA, 1 mM Mg^{2+} solution. Portion of nuclei with nuclear envelope (NE) and chromatin fibrils (Ch). Arrows show 200 Å globules. (B) Nuclei in 20 mM TEA solution. Arrows show 100 Å globules. (C) Nuclei in 20 mM TEA, 1 mM Mg^{2+} and 6 mM EDTA. Chromatin fibrils devoid of globular structure. (D) Chromatin after shadowing with palladium. Arrows show 200 Å globules. (E) Chromatin after negative staining with uranyl acetate. Arrows show 200 Å globules. (F) Globular chromatin after EDTA treatment. Negative staining with uranyl acetate. Arrows show 100 Å globules.

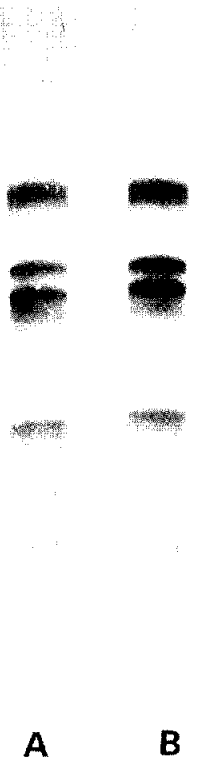

Fig.2. Polyacrylamide gel electrophoresis of histones. Histones from (A) isolated intact nuclei and (B) globular chromatin.

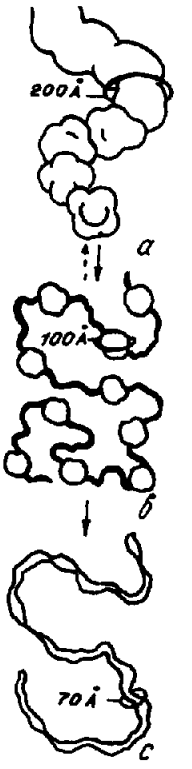

Fig.3. Possible structural levels of globular chromatin fibrils. (a) Basic structural unit of 200 Å globular chromatin when present both in intact nuclei and in chromatin fractions. (b) 200 Å → 100 Å structural transition of globular chromatin after partial removal of Mg^{2+} from the medium. (c) 100 Å → 70 Å structural transition after further removal of Mg^{2+} from chromatin by EDTA.

4. Discussion

On the basis of the data obtained, the organization of chromatin fibrils and their structural transitions may be schematically presented in the following way (fig.3). The basic structural unit of chromatin is a 200 Å DNP fibril consisting of stacked globules of the diameter.

The presence of 200 Å chromatin fibrils in situ, as seen in ultrathin sections and by the freeze-etching technique, allows one to suggest that the 200 Å fibril is a native chromatin structure. According to Brinkley [12], Onishchenko and Chentsov [13], the peripheral layer of chromatin consists of stacked 200 Å globules. Thus DNP globules are present in nuclei in situ, which also shows that they are genuine native structural entities of chromatin fibrils.

Recently some authors [1–4] have reported the detection of 100 Å spherical bodies in DNP fibrils. In our experiments such structures were present after removal of part of the Mg^{2+}. The 80–100 Å spherical bodies seem to occur when low ionic strength or magnesium-free solutions were used for isolation of chromatin. Mg^{2+} is likely, therefore, to be indispensable for both 200 Å globules and 100 Å spherical bodies to remain intact. At still lower Mg^{2+} concentration in the medium DNP becomes completely devoid of globules and only 50–70 Å fibrils are visible in the chromatin. It is noteworthy that the latter transformation may only be caused by removal of Mg^{2+} by EDTA. One may conclude that in chromatin there are both weakly bound Mg^{2+}

ions, that may be removed with water, and firmly bound Mg^{2+} ions for whose removal a chelating agent is required. When the weakly-bound Mg^{2+} ions are removed a DNP fibril containing 200 Å globules transforms into one consisting of 100 Å globules. Such a structural transition may be due either to the decrease in the diameter of an individual globule from 200–100 Å, or to the unfolding of such a globule into a chain of 100 Å globules. Since no release of protein from chromatin is observed in the transition from 200 Å to 100 Å globule, the second possibility appears to be more plausible. If this is so, it should be assumed that a 200 Å globule consists of at least six 100 Å globules.

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