STEREO ELECTRON MICROSCOPY OF THE 25-nm CHROMATIN FIBERS IN ISOLATED NUCLEI

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ABSTRACT

Thin sections (0.1–0.25 μm) of isolated chicken erythrocyte nuclei were examined at various tilt angles. Stereo pairs of electron micrographs document the parallel alignment of 25-nm chromatin fibers adjacent to the nuclear envelope, and demonstrate a fiber substructure consistent with close-packed arrays of nucleosomes.

KEY WORDS stereo electron microscopy · nucleosomes · higher-order structure · chromatin structure · nuclei

The condensed regions of eukaryotic chromatin have been shown to consist of unit threads of nucleohistone ~25 nm in diameter (5, 6, 24, 28). Since the discovery of the nucleosomes (8, 10, 16–18, 23, 26), there have been attempts to visualize and interpret the unit thread as: helical arrays of close-packed nucleosomes (3, 4, 11, 12); a hollow tube (solenoid) of a helically coiled nucleofilament (7); or close-packed clusters (superbeads) composed of nucleosomes (9, 12, 21). Ultrastructural studies on thin sections cut tangential to chicken erythrocyte nuclei have revealed parallel arrays of unit threads adjacent to the nuclear envelope (6). The 25-nm chromatin fibers are maintained in isolated nuclei, in appropriate solvent conditions, and can be visualized in thin sections or by spreading techniques (12, 15, 20). In the present study, we have employed stereo electron microscopy on sections of slightly swollen isolated chicken erythrocyte nuclei. This has enabled us to follow unit threads over considerable distances within the nuclei and to visualize the parallel arrays adjacent to the nuclear envelope. Furthermore, the improved signal-to-noise ratio inherent in binocular vision has permitted us to visualize the substructure of the 25-nm fibers as close-packed arrays of nucleosomes.

MATERIALS AND METHODS

Sections (0.10–0.25 μm thick) were obtained from the same blocks of glutaraldehyde- plus osmium-tetroxide-fixed chicken erythrocyte nuclei that were employed in a previous study (15). At that time, we observed that nuclei swollen in 20 mM KCl, 1 mM cacodylate (pH 7.5) before and during fixation revealed excellent preservation and separation of unit threads. These slightly swollen nuclei form the basis of the data presented in this investigation.

Carbon-coated parloidon films were used to support thin sections of nuclei which were stained with 2% uranyl magnesium acetate at 54°C for 20 min and counter-stained with lead citrate (22).

A Siemens 102 electron microscope equipped with a "double-tilt lift" device was used to prepare the stereo pairs. Regions near the center of the grid were chosen to minimize magnification differences between pairs. The maximum difference in objective current setting for any two members of a pair was 0.88%, and most pairs varied by only 0.1–0.2%. The pairs were chosen from a series of micrographs at different tilt angles so as to give the best depth perception with minimum eye strain.

RESULTS

Isolated eukaryotic nuclei undergo rapid morphological changes when treated with buffers of varying pH and electrolyte concentration (1, 19, 25). We have previously shown (15) that isolated chicken erythrocyte nuclei swell when diluted into 20 mM KCl (pH 7.5): the unit threads separate from one another, but the fiber diameters remain

comparable to their values in more condensed nuclei (i.e., in 200 mM KCl). Furthermore, 25-nm chromatin fibers revealing close-packed arrays of nucleosomes are observed at the periphery of nuclei swollen in 20 mM KCl and centrifuged onto carbon-coated grids and negatively stained (12). Lower ionic strengths (e.g., 1 mM) result in a greater degree of swelling and rupturing of nuclei: the unit threads unravel and reveal fibers 5–10 nm wide in thin sections (2, 14) and the characteristic "beads-on-a-string" in spread preparations (13, 14, 17).

The separation of 25-nm fibers in nuclei in 20 mM KCl has permitted us to do tilting studies on sections without too much image confusion from overlapping structures, even with nonspecific staining (i.e., uranyl ions). Fig. 1 is a stereo pair of a 0.25-μm-thick section revealing a tangential slice of a nucleus in the top portion of the figure and a section through the middle of a nucleus in the bottom portion. The unit threads are only clearly visualized in the tangential slice. To study

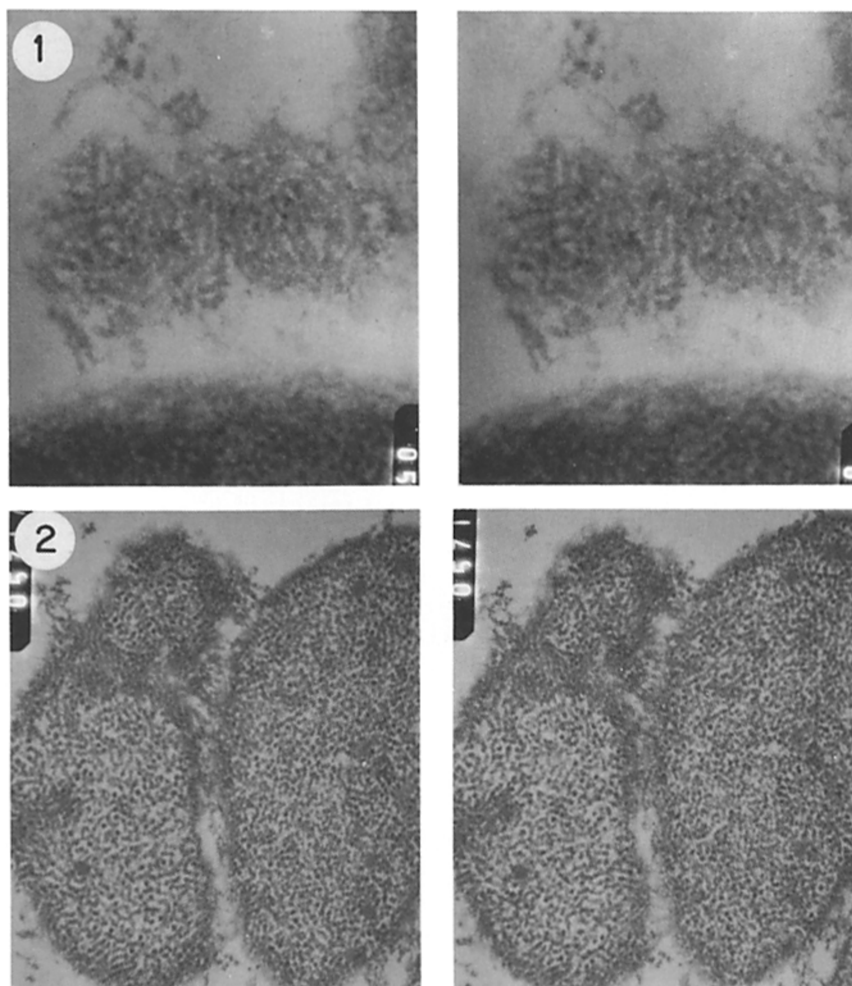


FIGURE 1 Section (0.25 μm thick) tangential to the surface of a chicken erythrocyte nucleus. At the bottom of the figure is a small portion of a nucleus which was sectioned through a more central plane and which clearly shows the depth of the entire section. The 25-nm fibers lie parallel to each other near the surface of the nucleus. Tilt angle, ±4°; × 56,000.

FIGURE 2 Section (0.1 μm thick) revealing two swollen erythrocyte nuclei in close proximity. Tilt angle, ±20°; × 28,000. The reader can avoid image confusion when looking at Figs. 1 and 2 by masking all but the figure of interest with white paper.

chromatin fibers throughout the nucleus, with the minimum of overlapping detail, we have confined most of our investigations to sections ~0.1 μm thick. Fig. 2 is a stereo pair of two nuclei in close proximity; the left nucleus shows particularly clear parallel arrays of 25-nm fibers within an indentation of the nuclear surface and at the bottom portion of the nucleus.

Employing stereo microscopy, we have been able to follow some unit threads for considerable distances, observing loops and bends as they cross through the swollen nuclei. Fig. 3 clearly demon-

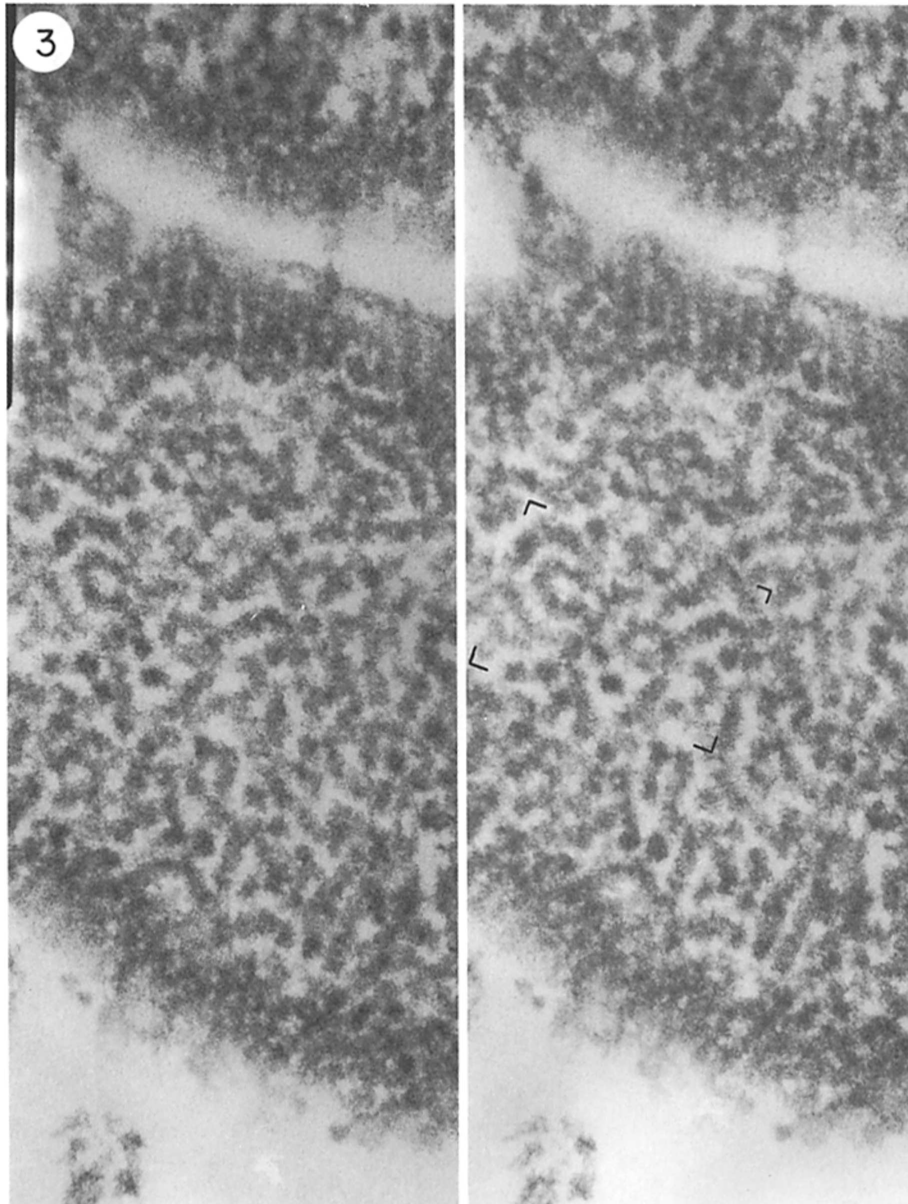


FIGURE 3 Section (0.1 μm thick) passing through a central portion of a nucleus. The 25-nm fibers near the surface of the nucleus show clear parallel alignment. Also apparent are looped chromatin fibers and apparent close packing of nucleosomes within the 25-nm fibers (brackets). Tilt angle, ±10°; × 112,000.

strates a looped fiber as well as the parallel alignment of unit threads adjacent to the nuclear envelope. The center-to-center spacing of the parallel unit threads (108 measurements) was found to be 31.7 ± 4.4 nm (±SD). Measurements of the stained fiber widths in several such photographs yielded an average (104 measurements) of 23.5 ± 3.8 nm.

At the magnifications presented here, Figs. 3 and 4 exhibit apparent substructure in the 25-nm fibers. Enlarged selected regions of Fig. 4 are presented in Figs. 5 and 6. When viewed in stereo, many localized regions appear to consist of close-packed spheroid objects not apparent in the sur-

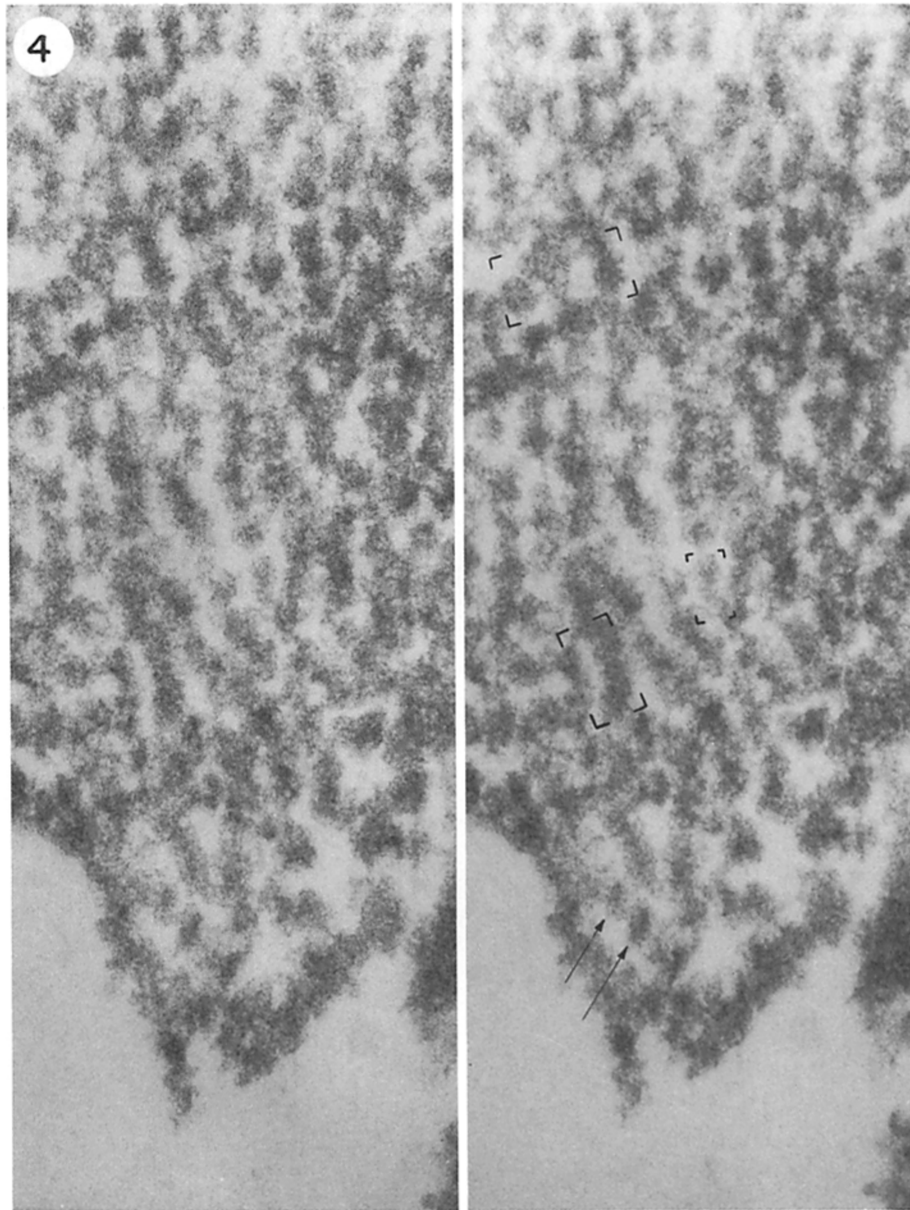
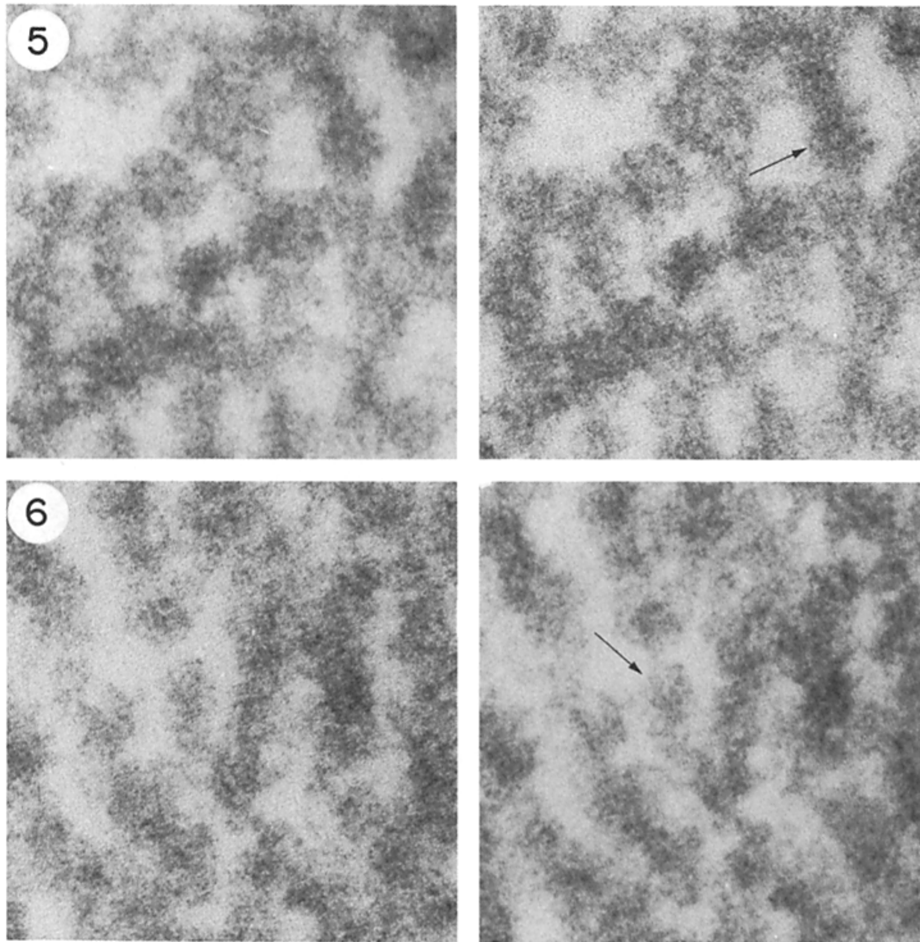


FIGURE 4 Section of chicken erythrocyte nuclei which apparently demonstrates close packing of nucleosomes in the 25-nm chromatin fibers (brackets). A possible helical chromatin fiber is also shown (arrows). Tilt angle, ±5°; × 185,000.



FIGURES 5 and 6 Enlarged regions of Fig. 4, illustrating apparent close packing of nucleosomes in the 25-nm fiber (arrows). Tilt angle, ±5°; ×370,000.

rounding embedding media. These objects appear to be ~10 nm in diameter. We believe that they represent close-packed nucleosomes that have survived the fixation, dehydration, embedding, and staining procedures. In these preparations the arrangement of the presumptive nucleosomes is apparently quite polymorphic—they exhibit clustered and possibly helical regions. It would be difficult to prove that these structures are nucleosomes. At this point, it can only be argued that the substructure of these 25-nm fibers observed in thin sections of isolated erythrocyte nuclei, swollen in 20 mM KCl buffer, look remarkably like 25-nm fibers observed in fresh spreads of erythrocyte nuclei exposed to similar solvent conditions (12).

DISCUSSION

By employing stereo electron microscopy, we are able to obtain a more realistic conception of the three-dimensional organization of chromatin fibers in swollen nuclei, as well as to utilize the signal-to-noise ratio enhancement of binocular vision to visualize substructure in the 25-nm fibers. High resolution information is maintained by working with relatively thin sections (0.1–0.25 μm).

To our knowledge, no one has yet succeeded in obtaining long stretches of 25-nm chromatin fibers with a clear arrangement of nucleosomes into a particular type of helical or clustered regular structure. There are two major possible explanations

that must be considered: regular nucleosomal arrays within the 25-nm fibers are easily perturbed by the techniques required for electron microscopy; and/or in vivo polymorphism of the higher-order structure exists and probably reflects localized differences in nonhistone composition, histone modification, or nucleosome phasing.

When isolated chicken erythrocyte nuclei are swollen at low ionic strength (i.e., 20 mM KCl), the 25-nm chromatin fibers separate from one another yielding less overlap of structure, a distinct advantage for stero electron microscopy. It is interesting that the peripheral layer of chromatin is the most resistant to this dispersing effect since it has previously been suggested that this layer of chromatin is anchored to the inner nuclear membrane (27).

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