Visualization of Nucleosomes in Thin Sections by Stereo Electron Microscopy

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ABSTRACT Nucleosomes (~10 nm diameter) were clearly visualized in thin sections (~0.1 μm thick) of isolated chicken erythrocytes. The cells were lysed and fixed in low ionic strength buffers that maintained the chromatin as dispersed filaments and prevented the reformation of supranucleosomal structures. Stereo electron micrographs at high magnification demonstrate the stability of nucleosome structure in the dispersed chromatin state during fixation, dehydration, and embedding.

The nucleosomal structure of chromatin is most readily visualized by spreading or centrifuging dispersed nuclear preparations onto an electron microscope grid, followed by staining and/or metal shadowing (6–8, 16). Demonstration of nucleosomes in embedded, sectioned, and stained nuclear preparations is considerably more difficult. We have previously argued that stereo electron microscopy (stereo EM) of 25-nm chromatin fibers reveals a substructure consistent with close-packed arrays of nucleosomes (9). Thin-section microscopy of crystals (3) and paracrystalline arrangements (2) of core mononucleosomes has shown convincing evidence of the stability of isolated nucleosomes to procedures used for preparing thin sections for electron microscopy. Surprisingly, however, there are no reports of the identification of nucleosomes on chromatin filaments in thin section. Therefore, it remains an open question whether the nucleosomal structure of dispersed chromatin is maintained throughout the fixation, dehydration, embedding, and staining steps, particularly in view of the sensitivity of chromatin structure to ethanol dehydration and critical-point drying (13, 16). In the present study we present stereo electron micrographs that demonstrate dispersed chromatin consisting of mostly extended chains of nucleosomes in conventionally fixed, embedded, and sectioned chicken erythrocyte chromatin.

MATERIALS AND METHODS

Fresh chicken blood was collected in physiologic saline containing 25 U of heparin/ml. Erythrocytes were collected by centrifugation for 5 min at 100 *g*, and washed twice with saline by centrifugation. The cells were lysed and the chromatin was dispersed by briefly incubating the erythrocyte pellet at 4°C in a large volume (approx. × 400) of 1 mM Tris-HCl, pH 7.2, containing 2 mM EDTA. The swollen cells were gently homogenized with only one stroke in a loosely fitting glass-Teflon Potter-Elvejhem homogenizer, and layered over a 0.1-M sucrose solution containing the same concentration of Tris-HCl buffer and EDTA. The swollen cells were pelleted by centrifugation (30 min at 3,500 *g*) at 4°C. The supernate was discarded and the cellular pellet was fixed with 2.5% glutaraldehyde buffered with 3.5 mM sodium phosphate (pH 7.2), at 4°C for 30 min. This step was critical as buffers containing higher salt concentrations and low amounts of divalent cations (e.g., above 50 nM $MgCl_2$) resulted in recondensation of nucleosomal chains into higher order arrays (1, 5, 15, and footnote[1]). Osmication, dehydration, embedding, and sectioning were carried out as described previously (4). Gold sections (~0.1 μm thick) were stained at 22°–25°C for 15 min in 2% uranyl acetate dissolved in methanol, washed in methanol, and contrasted with lead citrate (14). A Philips 400 electron microscope equipped with a goniometer was used to obtain the stereo micrographs. No differences in objective lens current was measured for the two members of a stereo pair. Mounting and analysis of stereo pairs of electron micrographs was carried out as described (9, 12).

RESULTS AND DISCUSSION

Sections (~0.1 μm thick) of avian erythrocytes briefly lysed in low ionic strength buffers were examined by electron microscopy. At low magnification (Fig. 1), one observes that the entire plasma membrane ghost is filled with dispersed chromatin. Remnants of the nuclear envelope can be seen with chromatin material penetrating in multiple locations. The erythrocyte membrane, on the other hand, reveals less rupturing and does not permit the chromatin to disperse into extracellular space.

When these sections were examined at high magnification and tilted for stereo electron microscopy, the chromatin filaments exhibited a distinctly punctate substructure reminiscent of the characteristic zig-zag appearance of spread polynucleosomal strands (Fig. 2). The spaces between these chromatin filaments looked extremely electron-transparent, a situation

[1] Zentgraf, H., U. Müller, and W. W. Franke. Reversible *in vitro* packing of nucleosomal filaments into globular supranucleosomal units in chromatin of whole chick erythrocyte nuclei. *Eur. J. Cell Biol.* In press.

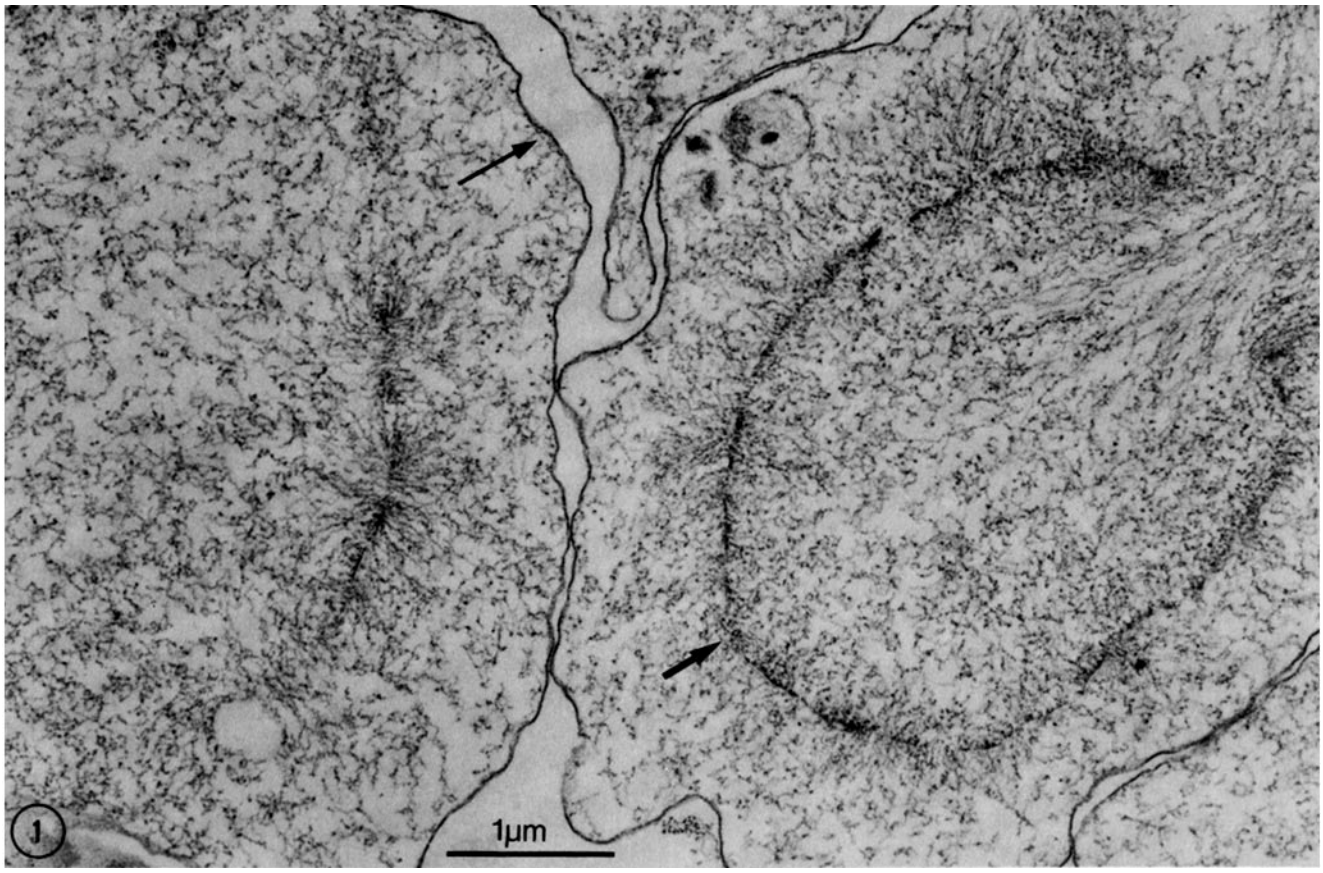


FIGURE 1 Survey electron micrograph of swollen chicken erythrocytes demonstrating the dispersal of chromatin throughout the cell. Thick arrow: remnants of nuclear envelope; thin arrow: erythrocyte plasma membrane. × 22,000.

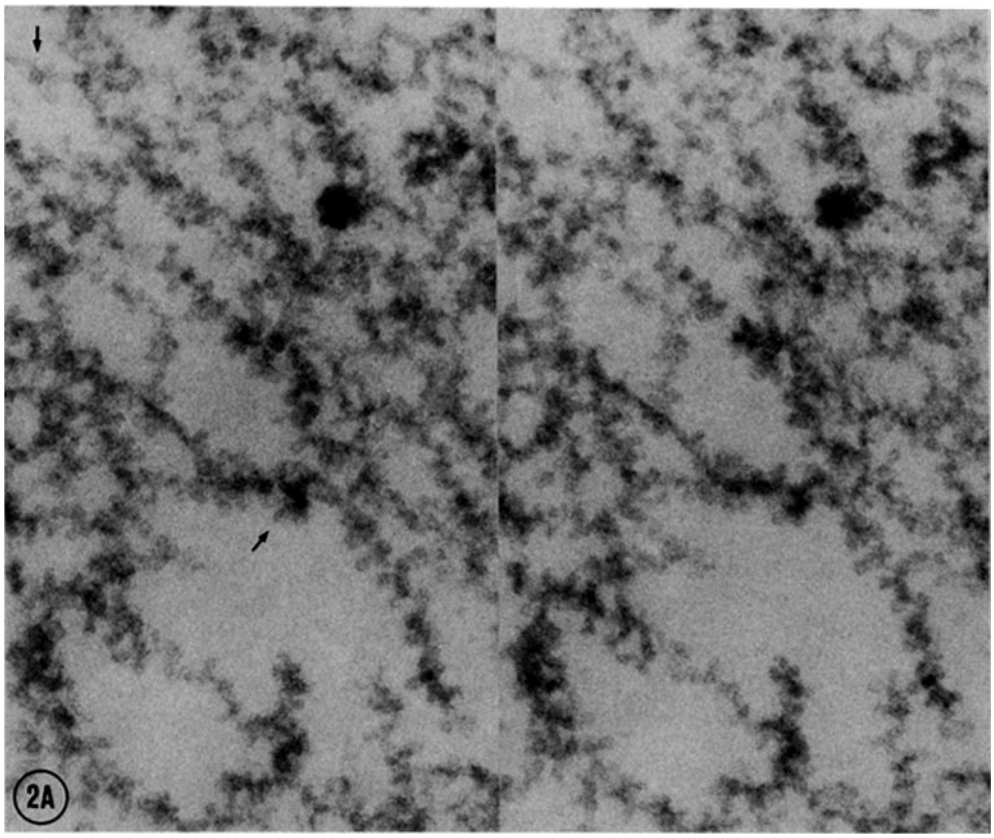
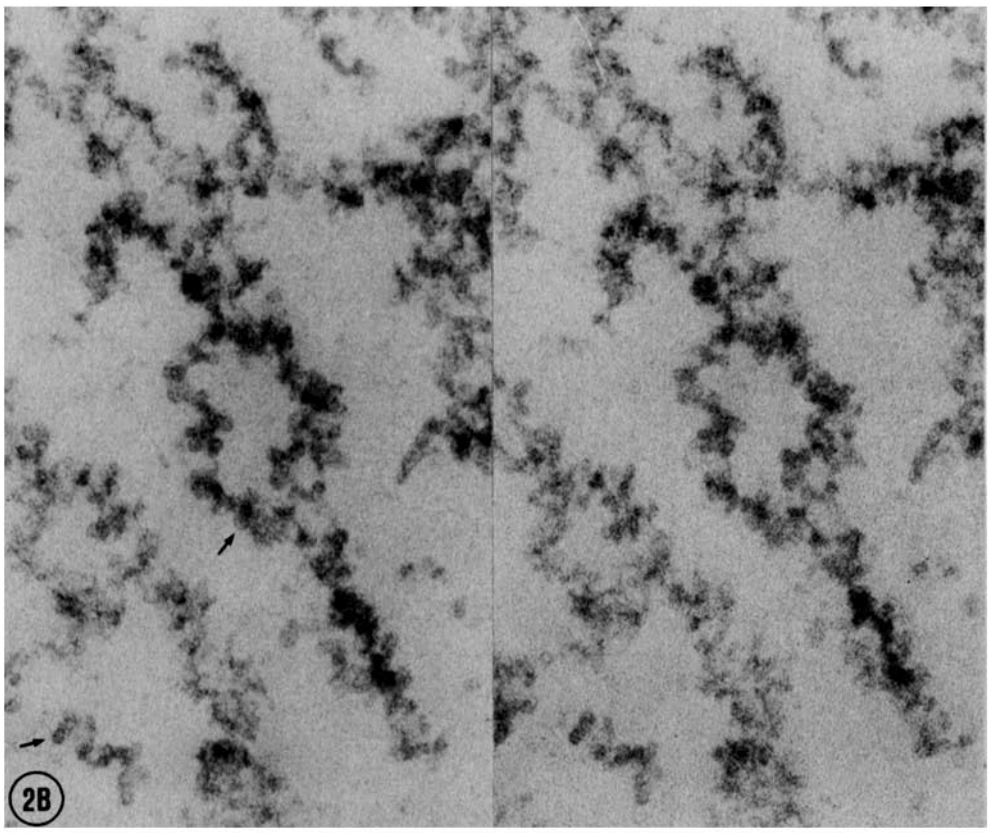
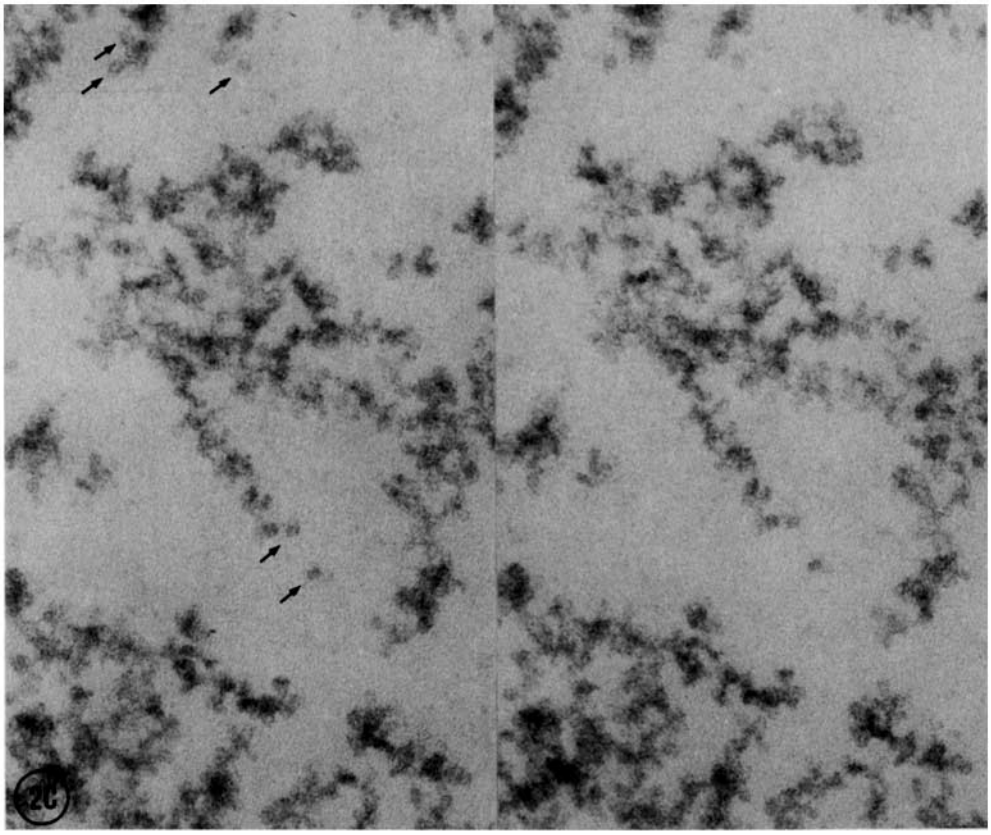


FIGURE 2 (*A–C*) High-resolution stereo micrographs illustrating the nucleosomal structure of dispersed erythrocyte chromatin. Arrows: particles with clear internal structure. × 194,000. Stereo tilt angle, ± 6°.





that facilitated both optimal stereo perception and visualization of structures at high resolution.

Scattered throughout the field of view, nucleosomal chromatin could be observed in varying degrees of clarity. Measurements of the diameter of these particles yielded an average of 9.5 ± 0.8 nm (SD), a value very close to measured diameters of lightly stained isolated mononucleosomes (10). Some of the particles observed by stereo EM exhibited internal structure with stain localized in the center of the particle, also reminiscent of the internal structure of lightly stained mononucleosomes and spread "beads-on-a-string" (11). At these low salt conditions, no regular higher order supranucleosomal structures remained.

The unmistakable identification of nucleosomal structures in fixed, dehydrated, and embedded chromatin presented in this study is caused by several conditions: (*a*) The fixation was carried out at low ionic strength and in the absence of divalent cations, thus preventing the maintenance of 20- to 30-nm supranucleosomal structures. (*b*) The swelling conditions at low salt and the physical barrier of the plasma membrane ghost prevented excessive stretching of the chromatin filament. (*c*) High-magnification stereo electron microscopy and the apparent absence of nonchromatinous material permitted us to obtain very clear nucleosomal images.

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