Visualization of Nucleosomes in Thin Sections by Stereo Electron Microscopy

ADA L. OLINS, DONALD E. OLINS, HANSWALTER ZENTGRAF, and WERNER W. FRANKE

Institute for Cell and Tumor Biology, and Institute of Virus Research, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany. Dr. A. L. Olins's and Dr. D. E. Olins's present address is The University of Tennessee—Oak Ridge Graduate School of Biomedical Sciences, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

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.

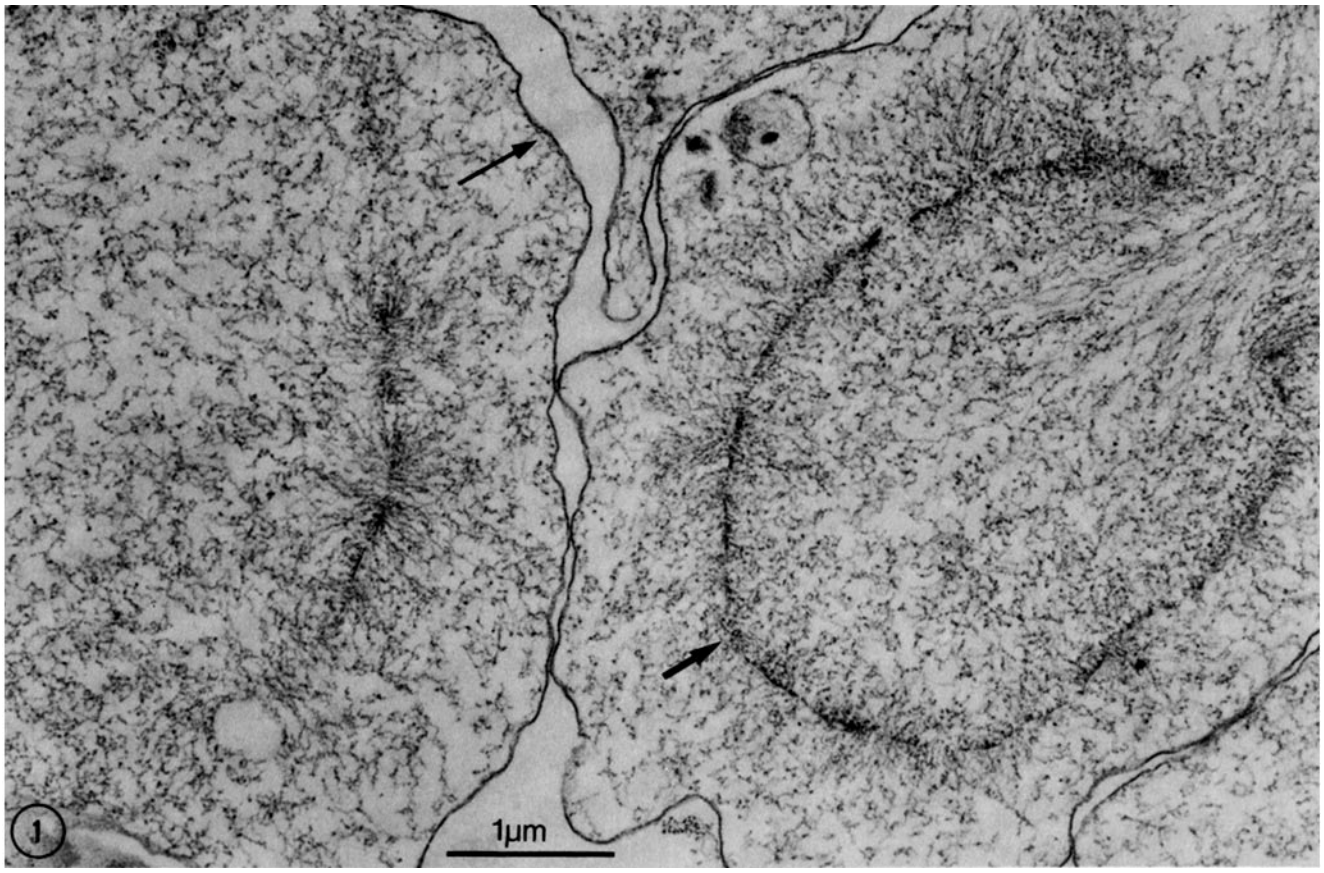


FIGURE 1 Survey electron micrograph of swollen chicken erythrocytes demonstrating the dispersal of chromatin throughout the cell. Thick arrow: remnants of nuclear envelope; thin arrow: erythrocyte plasma membrane. × 22,000.

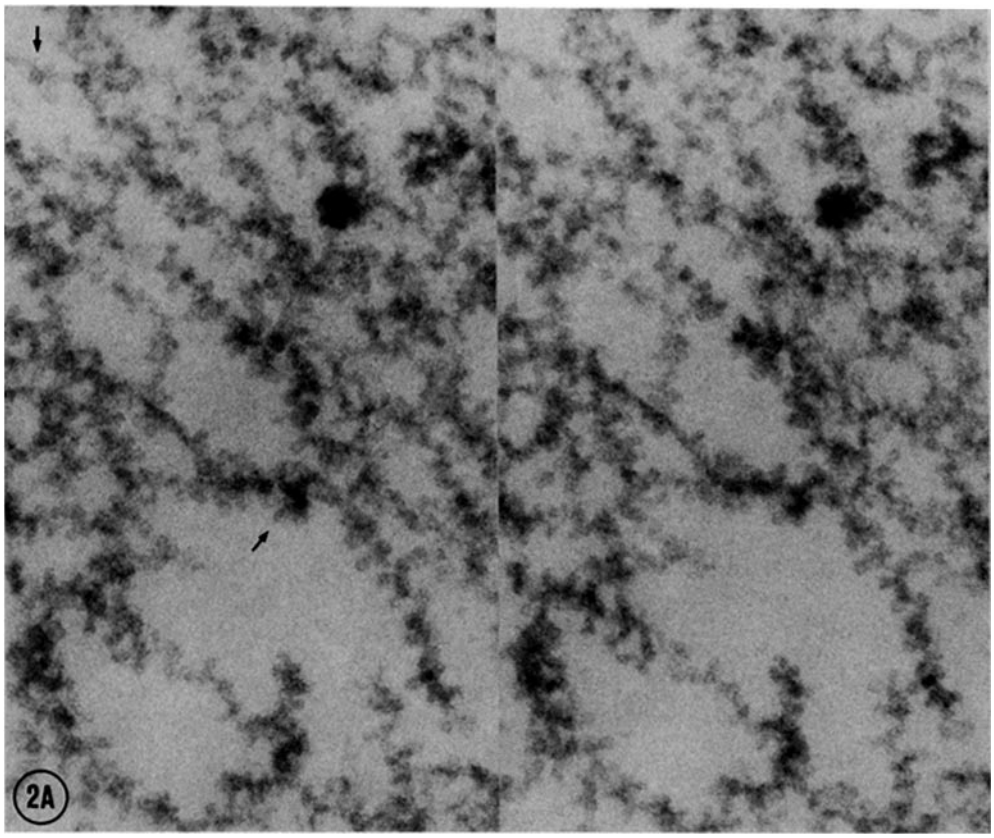
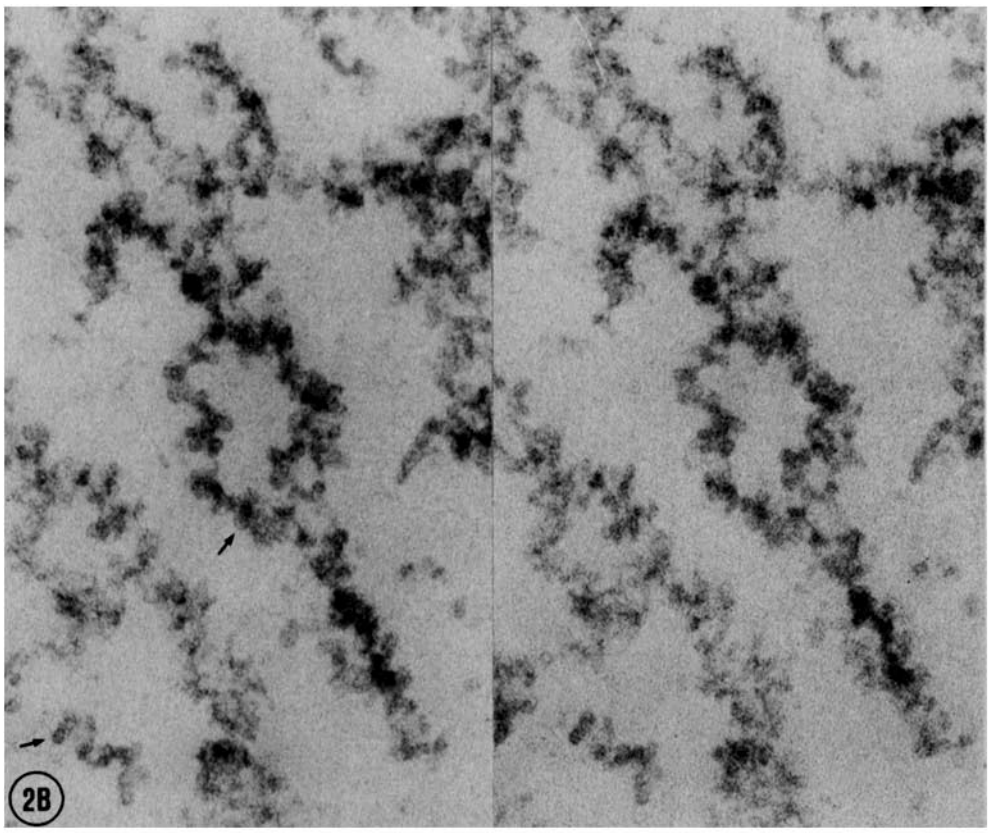
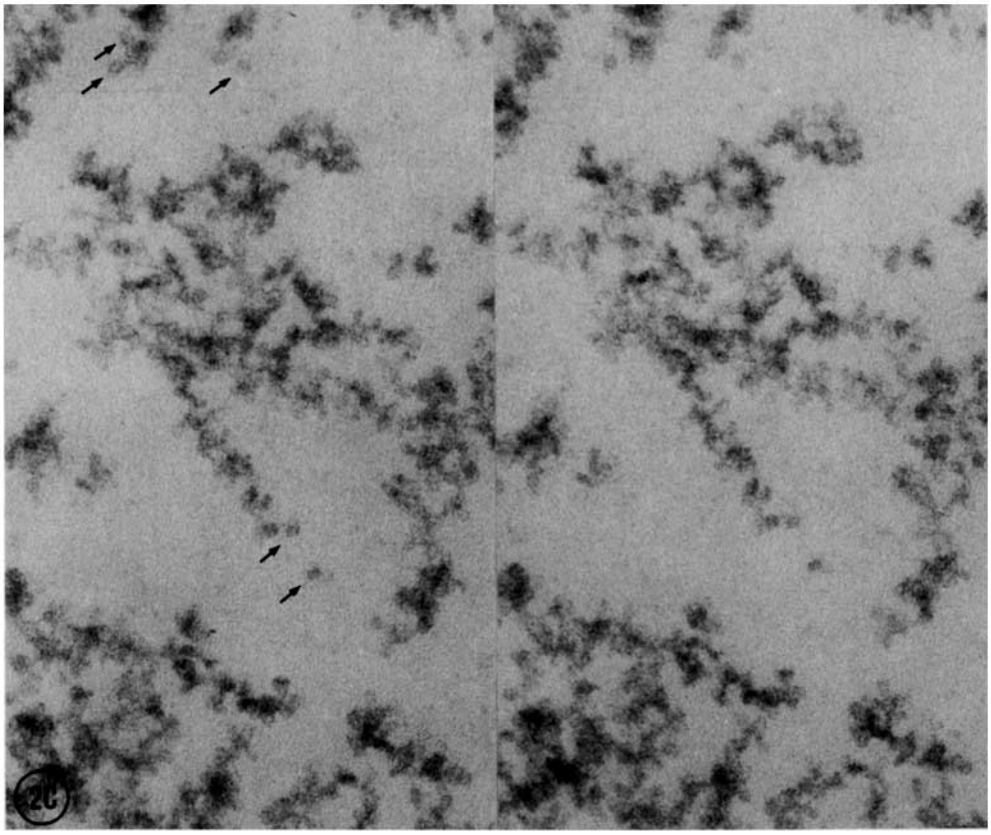


FIGURE 2 (*A–C*) High-resolution stereo micrographs illustrating the nucleosomal structure of dispersed erythrocyte chromatin. Arrows: particles with clear internal structure. × 194,000. Stereo tilt angle, ± 6°.


2B


2C

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.

We thank Dr. N. Paweletz (German Cancer Research Center, Heidelberg) for permitting us to use a Philips 400 electron microscope and Mr. K. Mähler for excellent photographic assistance. Dr. D. E. Olins is a recipient of a Senior U. S. Scientist Award from the Alexander von Humboldt Foundation. This work has been supported by the Deutsche Forschungsgemeinschaft.

A. L. Olins is a Visiting Professor of the German Cancer Research Center.

Received for publication 28 July 1980.

REFERENCES

1. Brasch, K., V. L. Seligy, and G. Setterfield. 1971. Effects of low salt concentration on structural organization and template activity of chromatin in chicken erythrocyte nuclei. *Exp. Cell Res.* 65:61–72.
2. Dubochet, J., and M. Noll. 1979. Nucleosome arcs and helices. *Science (Wash. D. C.).* 202: 280–286.
3. Finch, J. T., and A. Klug. 1977. X-ray and electron microscope analyses of crystals of nucleosome cores. *Cold Spring Harbor Symp. Quant. Biol.* 42:1–9.
4. Franke, W. W., M. R. Lüder, J. Kartenbeck, H. Zerban, and T. W. Keenan. 1976. Involvement of vesicle coat material in casein secretion and surface regeneration. *J. Cell Biol.* 69:173–195.
5. Kiryanov, G. I., T. A. Manamshjan, V. Yu. Polyakov, D. Fais, and Ju.S. Chentsov. 1976. Levels of granular organization of chromatin fibres. *FEBS (Feb. Eur. Biochem. Soc.). Lett.* 67:323–327.
6. Olins, A. L., 1978. Visualization of chromatin ν-bodies. *In* Methods in Cell Biology. G. Stein and L. Kleinsmith, editors. Academic Press, Inc., New York. 18:61–68.
7. Olins, A. L. 1979. Electron microscopy: A tool for visualizing chromatin. *In* Chromatin structure and function. C. A. Nicolini, editor. Plenum Publishing Corp., New York. Pt. A. 31–40.
8. Olins, A. L., and D. E. Olins. 1974. Spheroid chromatin units (ν bodies). *Science (Wash. D. C.).* 183:330–332.
9. Olins, A. L., and D. E. Olins. 1979. Stereo electron microscopy of the 25-nm chromatin fibers in isolated nuclei. *J. Cell. Biol.* 81:260–265.
10. Olins, A. L., R. D. Carlson, E. B. Wright, and D. E. Olins. 1976. Chromatin ν-bodies: Isolation, subfractionation and physical characterization. *Nucleic Acids Res.* 3:3271–3291.
11. Olins, A. L., J. P. Breillatt, R. D. Carlson, M. B. Senior, E. B. Wright, and D. E. Olins. 1977. On nu models for chromatin structure. *In* The Molecular Biology of the Mammalian Genetic Apparatus. P. Ts'o, editor. Elsevier/North-Holland Biomedical Press, Amsterdam. 211–237. (Chap. 11).
12. Olins, A. L., E. Wilkinson-Singley, J. Bentley, and D. E. Olins. 1979. Stereo-electron microscopy and energy-dispersive X-ray analysis of avian reticulocytes. *Eur. J. Cell Biol.* 19:239–249.
13. Pooley, A. S., J. F. Pardon, and B. M. Richards. 1974. The relation between the unit thread of chromosomes and isolated nucleohistones. *J. Mol. Biol.* 85:533–549.
14. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell. Biol.* 17:208–212.
15. Thoma, F., Th. Koller, and A. Klug. 1979. Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell. Biol.* 83:403–427.
16. Woodcock, C. L. F., J. P. Safer, and T. E. Stanchfield. 1976. Structural repeating units in chromatin. *Exp. Cell Res.* 97:101–110.