

ARIEL COVER SHEET:

THIS ARTICLE IS SENT ON BEHALF OF THE ILL OFFICE AT UMASS AMHERST (AUM) FROM THE FIVE COLLEGE LIBRARY DEPOSITORY.

BORROWING LIBRARY: BBH

ILL NUMBER: 54727230

PATRON: Olins, Ada

PLEASE TELEPHONE 413-542-8903 OR EMAIL: bunker@fivecolleges.edu in the event of any problems with this transmission

NOTICE: This material may be protected by Copyright Law. (Title 17 U.S. Code)

Ida Hay

From: ILL/Lending [illdd@library.umass.edu]
Sent: Wednesday, June 10, 2009 11:24 AM
To: Bunker
Subject: Request from UMass/Amherst

Dear Depository Staff,

UMASS AMHERST (AUM) Interlibrary Loan has received a request from another library for the following item held at the Five College Depository.

Please supply ARTICLES directly to the borrowing institution shown below.
Please send LOAN requests to the UMass ILL office.

EMAIL update replies to ILLDD@library.umass.edu and refer to OUR TRANSACTION NUMBER:
651726

Journal Title: European journal of cell biology.

Vol: 27 No: 2
Month: Year: June 1982

Article Author:

Article Title: Laughlin TJ, Wilkinson-Singley E, Olins AL, et al.; Stereo-electron microscope studies of mitotic chromosomes from Chinese hamster ovary cells

Article Pages: 170-176

OR

Loan Author:
Loan Title:

LOCATION:

CALL NUMBER: UC270150

BORROWING LIBRARY: BBH

ILL NUMBER: 54727230

PATRON: Olins, Ada

ARIEL: 139.140.112.4

FAX: (207) 725-3083

ADDRESS TO MAIL OR FAX PHOTOCOPY IF NECESSARY:

Bowdoin College Library - ILL

3001 College Station
Brunswick, ME 04011-8421

Stereo electron microscope studies of mitotic chromosomes from Chinese hamster ovary cells

Tommie J. Laughlin¹), Elizabeth Wilkinson-Singley, Donald E. Olins, Ada L. Olins

The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN/USA

Received September 22, 1981

Accepted February 10, 1982

Stereo-electron microscopy — Chinese hamster ovary — mitotic chromosomes — chromosome isolation — chromatin

Stereo electron microscopy was employed to examine thin sections of Chinese hamster ovary metaphase chromosomes in situ and of chromosomes released from Chinese hamster ovary cells by several methods. Detergent lysis of cells in a buffer containing Mg^{++} and Ca^{++} and hypotonic lysis of cells in a hexylene glycol- Ca^{++} buffer released chromosomes that exhibited a three-dimensional meshwork of about 50 nm chromatin fibers. Fragmentation of cells in serum-free medium by vortexing with glass beads revealed a more dispersed chromosomal morphology with a mesh of 10 to 25-nm fibers exhibiting a presumptive nucleosomal substructure. Possible origins of the various fiber sizes are discussed in terms of current models of metaphase chromosome structure.

Introduction

Metaphase chromosome organization has received intense scrutiny by use of electron microscopy [5, 6, 18, 21]. Ultrastructural studies of metaphase chromosomes fixed in situ have been largely uninformative due to the dense packing of chromatin fibers. Consequently, ultrastructural studies have concentrated upon the morphological states of isolated metaphase chromosomes. This approach has focused importance on the various methods of cell lysis and chromosome isolation and the resulting diversity of chromatin fiber diameter and fiber folding. In this communication, we employ conventional and stereo transmission electron microscopy (stereo-EM) to visualize the three-dimensional arrangement of metaphase chromosome fibers after treatment with several established chromosome isolation techniques.

¹) Dr. T. J. Laughlin, Biology Division, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, Tennessee 37830/USA.

Materials and methods

Cell culture

Chinese hamster ovary (CHO) cells obtained from Dr. R. A. Tobey (Los Alamos Scientific Laboratory) were grown in monolayer stock cultures as described elsewhere [9]. The cells were blocked in mitosis by a 6-h colchicine (0.5 $\mu\text{g}/\text{ml}$) treatment. The mitotic cells were collected by mitotic selection methods [20]. For examination of metaphase chromosomes fixed in situ (i. e., intact mitotic cells fixed in growth medium), glutaraldehyde (Polysciences) was added to a suspension of mitotic cells to a final concentration of 1% fixative.

Techniques for releasing chromosomes

Three published methods were employed to release metaphase chromosomes from fragmented cells, namely, the techniques developed by Wray and Stubblefield [23], Adolph [1], and Rattner and Hamkalo [18].

In the Wray and Stubblefield method [23], mitotic cells were chilled on ice for 30 min, pelleted by centrifugation, resuspended in a hexylene glycol solution (1.0 M hexylene glycol, 0.5 mM CaCl_2 , 0.1 mM Pipes, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), pH 6.5), pelleted, resuspended in hexylene glycol solution, incubated at 37°C for 10 min, and gently passed twice through a 22-gauge needle. The released chromosomes in isolation buffer were immediately fixed by addition of glutaraldehyde to a final concentration of 1%.

As a second method of cell lysis, we employed "method 1" of Adolph [1]. Mitotic cells were chilled on ice for 30 min, pelleted, resuspended in Mg/Ca solution (50 mM NaCl, 5 mM HEPES, 5 mM MgCl_2 , 0.5 mM CaCl_2 , 0.1 mM PMSF, pH 7.4), pelleted, resuspended in Mg/Ca solution twice more, and transferred to a Dounce homogenizer (Wheaton, type A). After adding 20% NP-40 (Shell) to a final concentration of 0.5%, the cells were homogenized with 5 to 10 strokes. Sodium deoxycholate was added to give a final concentration of 0.1%, and the suspension was homogenized with 5 to 10 more strokes. The majority of the chromosomes were released at this point. The suspension of released chromosomes was immediately brought to 1% glutaraldehyde.

The method of Rattner and Hamkalo [18] involves mechanical breakage of cells and release of chromosomes in the absence of de-

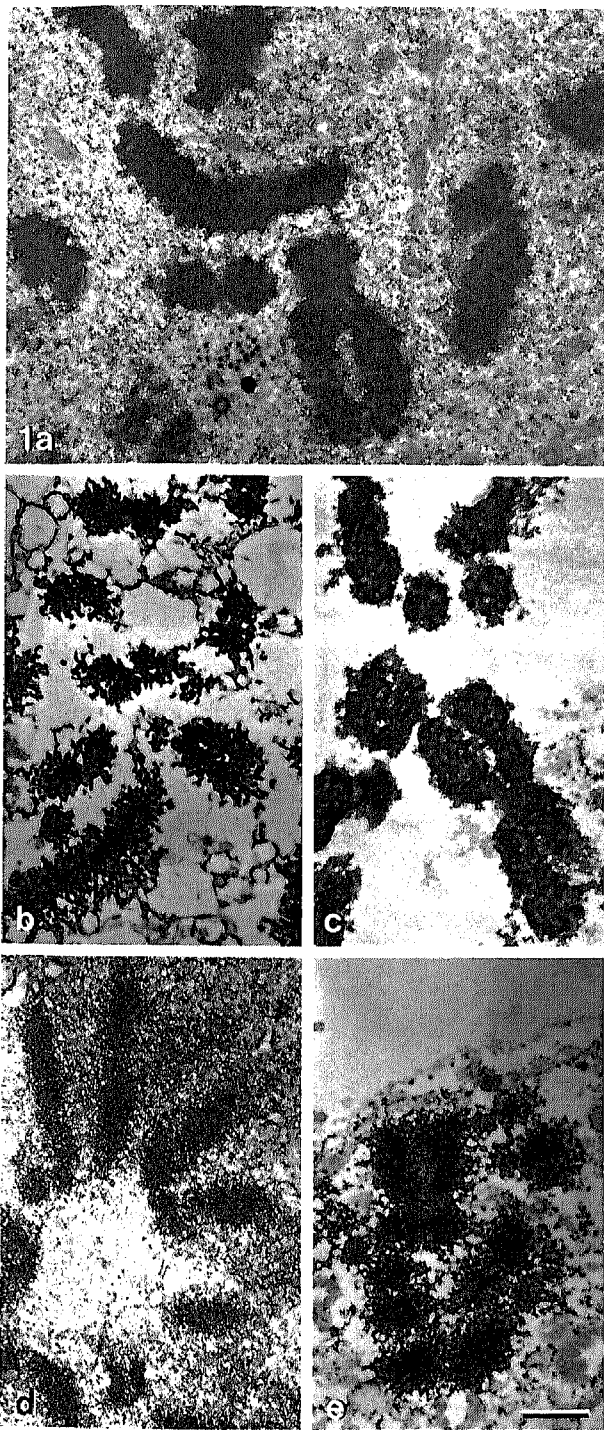


Fig. 1a to e. Survey electron micrographs of mitotic CHO cells and released metaphase chromosomes fixed under various buffer conditions. — a. Fixed in situ. — b. Released chromosomes fixed in the Wray and Stubblefield buffer. — c. Released chromosomes fixed in the Mg/Ca solution of Adolph. — d. Intact mitotic cell fixed immediately after exposure to the Mg/Ca solution. — e. Fragmented mitotic cell fixed in serum-free growth medium. — Bar 1 μm . — 8100 \times .

tergent. Mitotic cells were gently pelleted and resuspended in serum-free medium (Ham's F12 medium, GIBCO). Equal volumes of cell suspension and 0.5 mm diameter glass beads were vortexed for 15 sec at the maximum speed setting of a vortex mixer (Scientific Products S8223). Usually about half of the cells lysed at this point. Glutaraldehyde was immediately added to the solution to a final concentration of 1%.

Electron microscopy

For transmission electron microscopy, the released chromosomes or intact cells were pelleted after fixation for 1 to 5 h at 2°C. The pellets were diced and washed for 1 h in 0.1 M sodium cacodylate buffer (pH 7) at 4°C. In most cases, the blocks were postfixed for 1 to 2 h with 1% osmium tetroxide (Polysciences) in 0.1 M sodium cacodylate (pH 7.0) and subsequently washed for 1 h in the same buffer. The blocks were dehydrated through a graded ethanol series, embedded in Spurr's resin, cured, and sectioned. Sections (60 to 80 nm thick) were normally stained for 1 to 2 h with 5% uranyl magnesium acetate (Fisher) in 30% ethanol at 40°C, followed by post-staining with lead citrate [19]. Stereo-EM, mounting, and analysis of stereo pairs were performed as described previously [14].

Results

Conventional thin-section electron microscopy

Survey micrographs of the various metaphase chromosome states examined in this communication are presented in Figure 1. Note the qualitative differences in structure between metaphase chromosomes released by the Wray and Stubblefield (Fig. 1b) and by the Adolph (Fig. 1c) techniques. In the former method the thick chromatin fibers appear to extend outward from a dense central region, whereas the chromosomes prepared in the Mg/Ca solution appear as a complex network of fibers with no obvious central core. The cells briefly treated with the Mg/Ca solution and fixed prior to detergent lysis (Fig. 1d) exhibit mitotic chromosomes that have less contrast and are more dispersed than chromosomes fixed in situ (Fig. 1a). Cells fragmented by the Rattner and Hamkalo procedure (Fig. 1e) also exhibit a slightly more dispersed metaphase chromosome structure than the chromosomes of the in situ preparation.

Stereo-electron microscopy of mitotic chromosomes

A higher magnification stereo-pair of mitotic chromosomes fixed in situ is presented in Figure 2. Centrioles and a trilaminar kinetochore can be clearly seen. In the pericentriolar region virus-like particles characteristic of CHO cells [22] are readily apparent. Suggestions of substructure can often be observed within the metaphase chromosome arms. "Layers" of chromatin (Fig. 2) about 80 to 140 nm thick are seen. On the periphery of the chromosome we frequently see irregularly shaped protrusions with widths of 40 to 100 nm and spaced approximately 300 nm apart or at multiples of 300 nm. The difficulty in discerning chromosome ultrastructure from thin sections of mitotic cells fixed in situ is underscored in these micrographs.

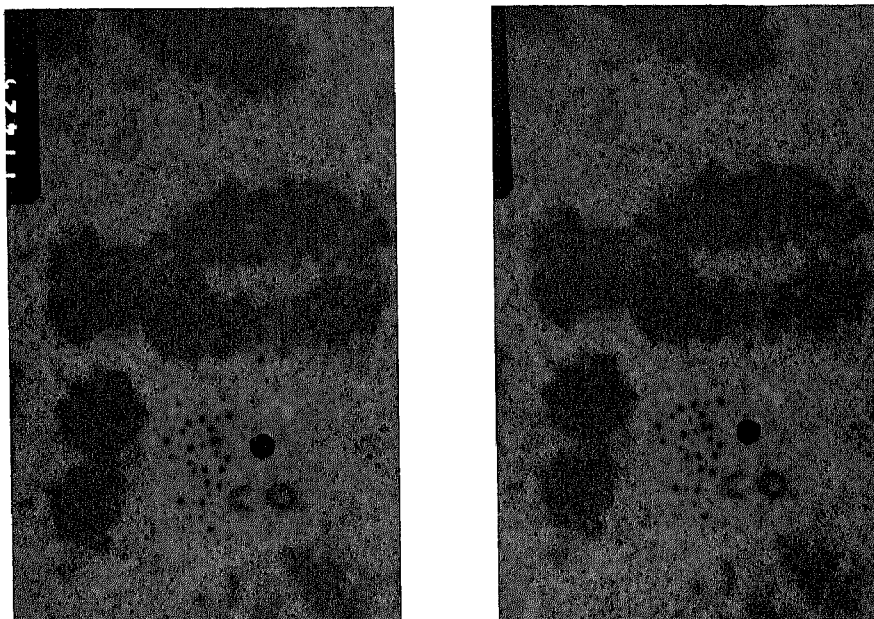


Fig. 2. Stereo electron micrographs of CHO metaphase chromosomes fixed in situ. One pair of chromatids is sectioned longitudinally; another pair appears to have been sectioned perpendicular to the long axis. The intense dark spot above the centrioles is an artifact. — Tilt angle $\pm 20^\circ$. — 15000 \times .

Metaphase chromosomes isolated by the Wray and Stubblefield technique exhibit a much more distinctly fibrous substructure (Fig. 3). The average fiber diameter is 53 ± 15 nm (Tab. I). This value agrees with the values of 50 to 60 nm reported by Marsden and Laemmli [10] and by

Adolph [1]. Generally, the density of chromatin fibers is relatively uniform within the central 400 nm of each chromosome arm. Outside of this region the density tends to decrease with distance from the chromatid axis. The chromatin fibers often appear to form loops in the outer regions.

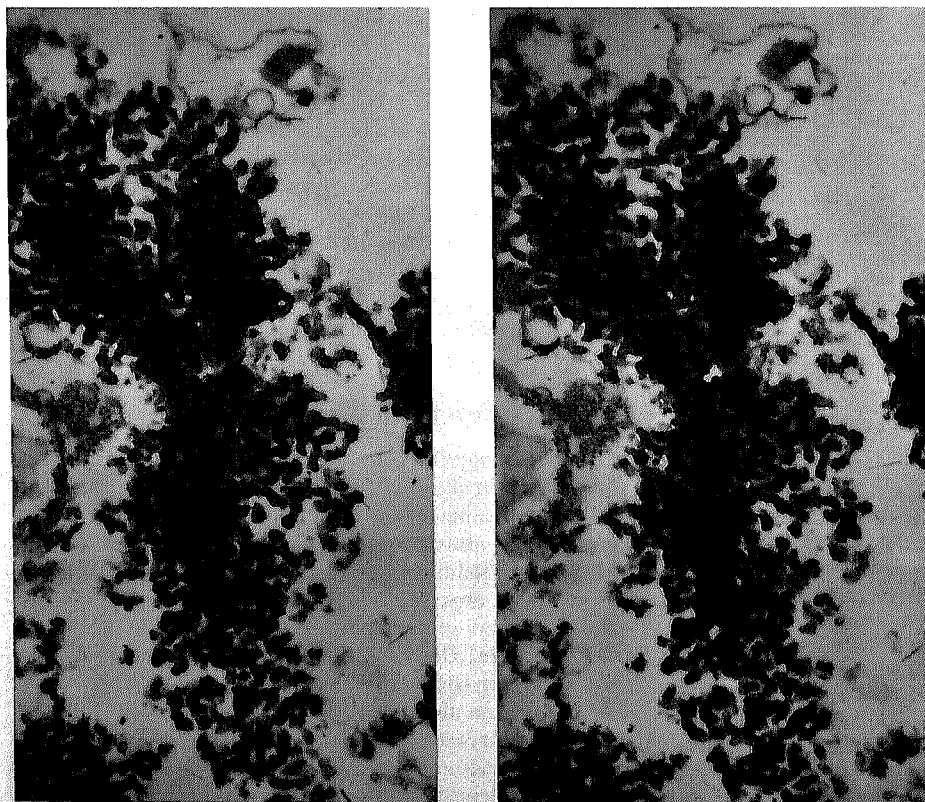


Fig. 3. Stereo electron micrographs of CHO metaphase chromosomes released from cells by the technique of Wray and Stubblefield into hexylene glycol solution. — Tilt angle $\pm 20^\circ$. — 25000 \times .

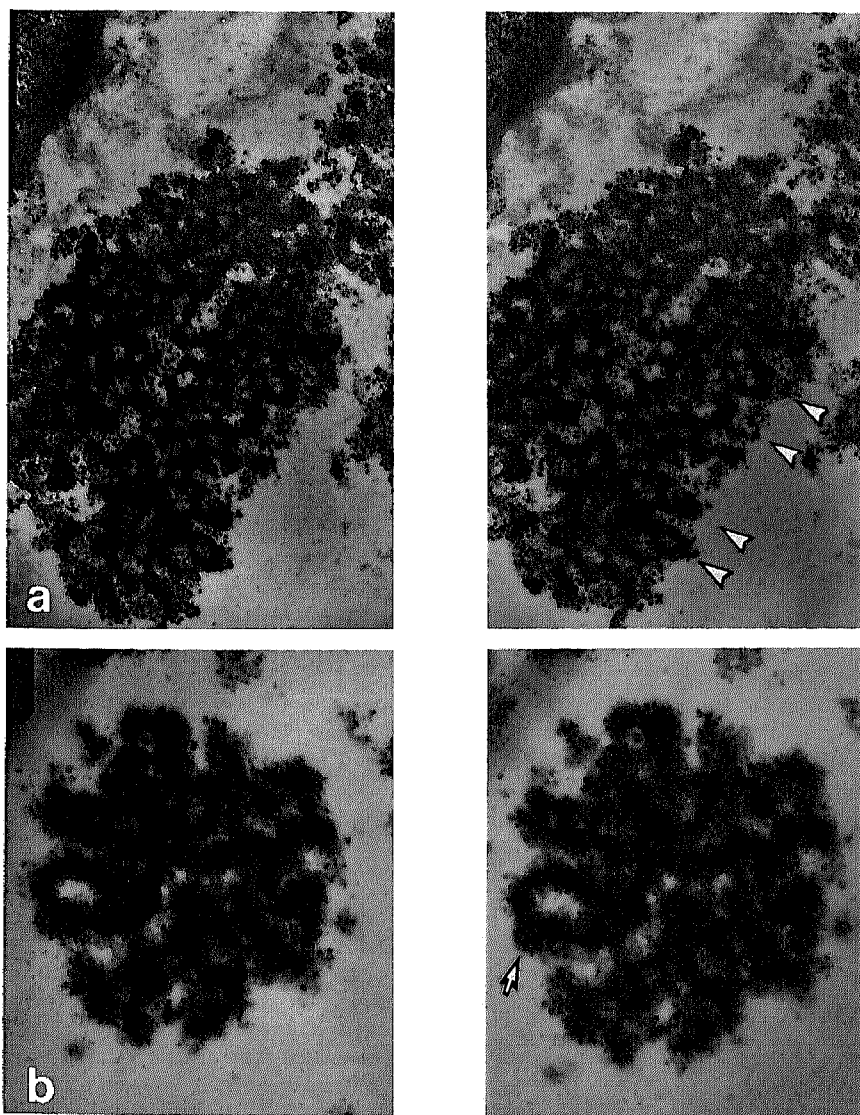


Fig. 4. Stereo electron micrographs of CHO metaphase chromosomes released from cells by the method of Adolph into a Mg/Ca solution. — **a.** Illustrates paired chromatids sectioned longitudinally. — **b.** Appears to have been sectioned perpendicular to the long axis. Note the apparent series of loops of 50 to 60 nm chromatin fibers at the lower right-hand edge of the chromatid in **a**, and the single large loop of an approximately 70 nm chromatin fiber on the left-hand edge of the chromatid in **b** (arrows). — **a.** Tilt angle $\pm 25^\circ$. — 2500 \times . — **b.** Tilt angle $\pm 20^\circ$. — 50 000 \times .

We observe fibers as long as 300 nm oriented both along the long axis of the chromatid and perpendicular to it.

A similar thick chromatin fiber structure is observed in metaphase chromosomes released into Mg/Ca solution employing method 1 of Adolph [1] (Fig. 4). The average fi-

ber diameter is 56 ± 15 nm (Tab. I). The chromatin density distribution is more uniform in this case. Occasionally, these fibers appear to form loops (Fig. 4). Chromosomes released by this method exhibit a significant contamination of electron dense granules (ca. 25 nm diameter) permeating the spaces between the thick chromatin fibers. These particles are probably related to the perichromosomal particles observed in situ when mitotic cells are stained by the Bernhard procedure [11, 13] and have been interpreted to be either hnRNP or ribosomes.

Metaphase chromosomes released from the cell by the method of Rattner and Hamkalo [18] reveal a more dispersed state consisting of a mesh of 18 ± 7 nm chromatin fibers (Fig. 5, Tab. I). A presumptive nucleosomal substructure can be observed with an apparent particle diameter of about 9.0 nm (data not shown). These 10 to 25-nm fibers (Tab. I) do not appear to be as uniform as those previously observed in metaphase chromosomes [17, 18].

Tab. I. Measurements of chromatin fiber diameters.

Isolation procedure	Mean (nm)	Standard deviation (nm)	Number of measurements
Rattner and Hamkalo (serum-free medium)	18	7	194
Wray and Stubblefield (hexylene glycol solution)	53	15	275
Adolph (Mg/Ca solution)	56	15	466
Adolph (prior to detergent lysis)	21	7	112

An even more dispersed metaphase chromatin organization is observed when intact mitotic cells are fixed immediately after exposure to the Mg/Ca solution (Fig. 6). This structural transition can also be readily observed in the phase light microscope: intact mitotic cells exposed to Mg/Ca solution exhibit a distinct loss of chromosome refractility. When the cell membrane is ruptured by detergent, the chromosomes rapidly condense. Ultrastructurally the dispersed chromatin exhibits a mesh of 21 ± 7 nm fibers (Tab. I) extensively permeated with presumptive ribonucleoprotein granules. It appears likely from these observations that rupture of the mitotic cells and release of the metaphase chromosomes by Adolph's method involves sequential stages of chromatin dispersion and recondensation.

Discussion

Several different levels of chromatin organization have been frequently observed in isolated metaphase chromosomes. Ten-nm fibers with the "beads-on-a-string" morphology have been reported [7, 17]. Chromatin fibers of 20 to 30-nm diameter have been repeatedly described and have been demonstrated to consist of a higher-order folding of nucleosomes [1, 6, 10]. Fibers of 50 to 60-nm diameter are often seen [1, 10, 13]. The substructure of the 50 to 60-nm fibers is the subject of continued controversy and the source of an abundance of models. Our data clearly indicate that the observed level of organization is a function of isolation procedure. We see 10 to 25-nm fibers when chromosomes are released from their cellular environment into serum-free growth medium (Fig. 5) and in the transi-

tion of in situ chromosomes to the condensed state induced by Mg/Ca solution (Fig. 6). The 50 to 60-nm fibers are observed in chromosomes stabilized by hexylene glycol or Mg/Ca solutions (Figs. 3, 4). The relation between the 50 to 60-nm fibers and lower levels remains unclear. Our evidence that intact mitotic cells exhibit a transient dispersion of metaphase chromosomes to about 20-nm fibers when exposed to hypotonic Mg/Ca-containing solutions suggests the possibility that the 50 to 60-nm fiber may represent a recondensed organization.

At this point our results do not enable us to present an independent model for chromosome fiber organization. Specific aspects of other models, however, can be reviewed in terms of our data. A few models regard chromosomes as very ordered coiled structures. Sedat and Manuelidis [16] proposed a "coiled-coils model" in which the 20 to 30-nm fiber is coiled into a very ordered helix to form a 200-nm tube which is coiled into a 600-nm chromatid. This model predicts a chromatin-free center of about 200-nm diameter. The "unit fiber model" [4] has proposed that the 20 to 30-nm fiber is helically coiled into a 400-nm tube with a hollow core. We have never seen such chromatin-free centers in our preparations, and generally the fiber arrangements are not as ordered as predicted by these models. "Folded fiber models" [5] assume a much less ordered packing of the chromatin fibers. Dupraw [5] suggests that the 20 to 30-nm chromatin fibers fold in reproducible, but irregular, patterns. Our micrographs can be interpreted in terms of this type of irregular packing. The "radial loops model" recently proposed by Laemmli and his colleagues [1-3, 8, 10, 15] suggests that 20 to 30-nm fibers are organized into radial loops anchored to an extensive protein scaffold. Recently, Mullinger and Johnson [12] presented evidence that

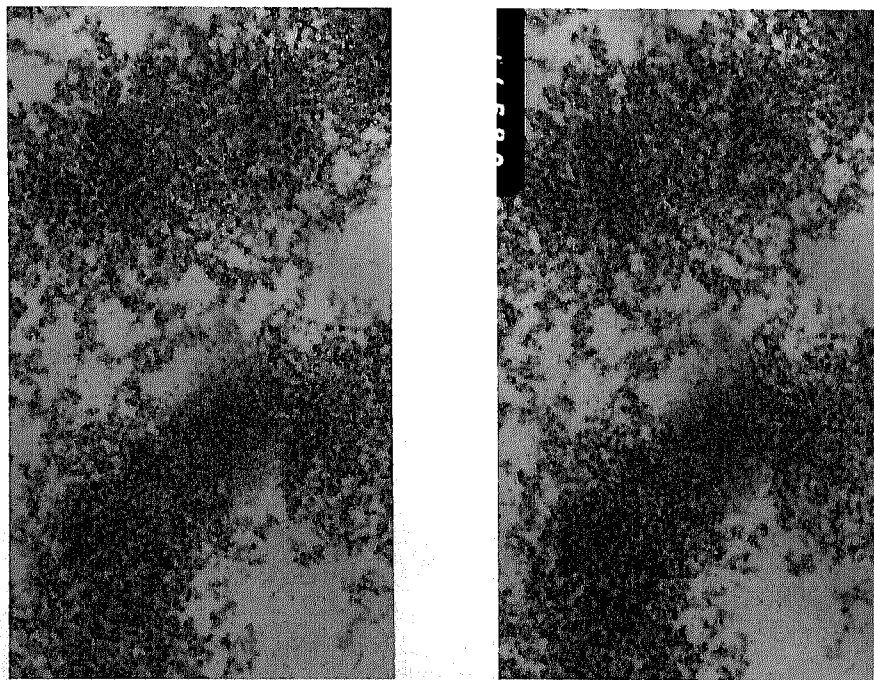


Fig. 5. Stereo electron micrographs of CHO mitotic cells fragmented by the technique of Rattner and Hamkalo, in serum-free growth medium. Note the kinetochore region, with remnants of the typical trilaminar structure, adjacent to the lower chromosome. — Tilt angle $\pm 25^\circ$. — 25 000 \times .

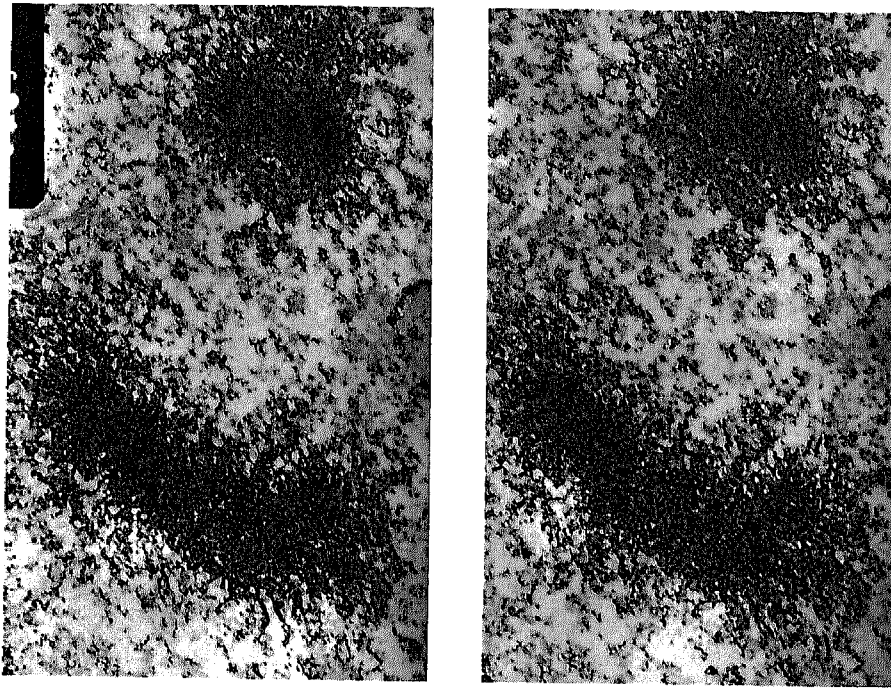


Fig. 6. Stereo electron micrographs of intact CHO mitotic cells treated and fixed in the Mg/Ca solution of Adolph, illustrating the highly dispersed state of these metaphase chromosomes. — Tilt angle $\pm 20^\circ$. — 25000 \times .

such radial loops may be organized about an axis which is helically coiled to form the chromatid. They suggest that a continuous DNA strand folds back on itself several times over short regions to form, in effect, a multistranded axis, and at a number of distinct foci the DNA emerges from the axis to form radial loops. We do not see compelling evidence for either a protein scaffold or an axial core, however, we do see what appear to be loops. We find the concept of radial loops organized about some sort of helically coiled axis particularly appealing since it can explain the phenomenon of macrocoiling (i.e., 200 to 400-nm fibers helically coiled to form a chromosome arm) reported in light and EM studies [5, 12].

Three-dimensional reconstruction of serial sections through mitotic chromosomes may eventually reveal the chromatin fiber organization. A recent analysis of serial sections of chromosomes from HeLa cells [2] suggests that there is a radial arrangement of such 20 to 30-nm fibers. We are currently employing computer-assisted three-dimensional reconstruction of tilted serial sections to attempt a clarification of the chromatin fiber arrangements within isolated mitotic chromosomes.

Acknowledgements. The authors express their appreciation to Dr. E. Uberbacher for helpful discussions and criticisms. We also thank Dr. R. J. Preston for generously allowing us access to the needed tissue culture facilities, and M. Hsie-Hsu for excellent photographic assistance. This research was supported jointly by grants from the National Institutes of Health (GM 19334), the National Science Foundation (PCM 21498), and the Office of Health and Environmental Research, Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.

References

- [1] Adolph, K. W.: Organization of chromosomes in mitotic HeLa cells. *Exp. Cell Res.* **125**, 95-103 (1980).
- [2] Adolph, K. W.: A serial sectioning study of the structure of human mitotic chromosomes. *Eur. J. Cell Biol.* **24**, 146-153 (1981).
- [3] Adolph, K. W., S. M. Cheng, J. R. Paulson, U. K. Laemmli: Isolation of a protein scaffold from mitotic HeLa cell chromosomes. *Proc. Natl. Acad. Sci. USA* **74**, 4937-4941 (1977).
- [4] Bak, A. L., G. Zeuthen: Higher-order structure of mitotic chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 367-377 (1977).
- [5] DuPraw, E. J.: *DNA and Chromosomes*, pp. 132-160. Holt, Rinehart & Winston, Inc., New York 1970.
- [6] Hamkalo, B. A., J. B. Rattner: VI. Folding up genes and chromosomes. *Q. Rev. Biol.* **55**, 409-417 (1980).
- [7] Howze, G. B., A. W. Hsie, A. L. Olins: v-Bodies in mitotic chromatin. *Exp. Cell Res.* **100**, 424-428 (1976).
- [8] Laemmli, U. K., S. M. Cheng, K. W. Adolph, J. R. Paulson, J. A. Brown, W. R. Baumbach: Metaphase chromosome structure: The role of nonhistone proteins. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 351-360 (1977).
- [9] Laughlin, T. J., J. H. Taylor: Initiation of DNA replication in chromosomes of Chinese hamster ovary cells. *Chromosoma* **75**, 19-35 (1979).
- [10] Marsden, M. P. F., U. K. Laemmli: Metaphase chromosome structure: Evidence for a radial loop model. *Cell* **17**, 849-858 (1979).
- [11] Moyne, G., J. Garrido: Ultrastructural evidence of mitotic perichromosomal ribonucleoproteins in hamster cells. *Exp. Cell Res.* **98**, 237-247 (1976).

- [12] Mullinger, A. M., R. T. Johnson: Packing DNA into chromosomes. *J. Cell Sci.* **46**, 61-86 (1980).
- [13] Okada, T. A., D. E. Comings: A search for protein cores in chromosomes: Is the scaffold an artifact? *Am. J. Hum. Genet.* **32**, 814-832 (1980).
- [14] Olins, A. L., D. E. Olins: Stereo-electron microscopy of the 25-nm chromatin fibers in isolated nuclei. *J. Cell Biol.* **81**, 260-265 (1979).
- [15] Paulson, J. R., U. K. Laemmli: The structure of histone-depleted metaphase chromosomes. *Cell* **12**, 817-828 (1977).
- [16] Sedat, J., L. Manuelidis: A direct approach to the structure of eukaryotic chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 331-350 (1977).
- [17] Rattner, J. B., A. Branch, B. A. Hamkalo: Electron microscopy of whole mount metaphase chromosomes. *Chromosoma* **52**, 329-338 (1975).
- [18] Rattner, J. B., B. A. Hamkalo: High order structure in metaphase chromosomes. II. The relationship between the 250 Å fiber, superbeads, and beads-on-a string. *Chromosoma* **69**, 373-379 (1978).
- [19] Reynolds, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208-212 (1963).
- [20] Stubblefield, E.: Synchronization methods for mammalian cell cultures. In: D. M. Prescott (ed.): *Methods in Cell Physiology*, Vol. III, pp. 25-43. Academic Press. New York 1968.
- [21] Stubblefield, E.: The molecular organization of mammalian metaphase chromosomes. In: F. E. Arrighi, P. N. Rao, E. Stubblefield (eds.): *Genes, Chromosomes and Neoplasia*, pp. 61-74. Raven Press. New York 1981.
- [22] Wheathley, D. N.: Pericentriolar virus-like particles in Chinese hamster ovary cells. *J. Gen. Virol.* **24**, 395-399 (1974).
- [23] Wray, W., E. Stubblefield: A new method for the rapid isolation of chromosomes, mitotic apparatus, or nuclei from mammalian fibroblasts at near neutral pH. *Exp. Cell Res.* **59**, 469-478 (1970).