NUCLEOSOME PACKING IN INTERPHASE CHROMATIN

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ABSTRACT

Higher-order chromatin fibers (200–300 Å in diameter) are reproducibly released from nuclei after lysis in the absence of formalin and/or detergent. Electron microscope analysis of these fibers shows that they are composed of a continuous array of closely apposed nucleosomes which display several distinct packing patterns. Analysis of the organization of nucleosomes within these arrays and their distribution along long stretches of chromatin suggest that the basic 100-Å chromatin fiber is not packed into discrete superbeads and is not folded into a uniform solenoid within the native 250-Å fiber. Furthermore, because similar higher-order fibers have been visualized in metaphase chromosomes, the existence of this fiber class appears to be independent of the degree of in vivo chromatin condensation.

KEY WORDS chromatin · higher order structure · 250 Å fibers · nucleosome packing

The elucidation of a particulate chromatin subunit, the nucleosome, as a ubiquitous component of eukaryotic chromatin has led to the investigation of the manner in which these subunits are organized into higher-order structures in both interphase and metaphase chromatin (1–4, 8–10, 12–14). Hozier et al. (5) reported that, in the presence of mono- or divalent cations, erythrocyte chromatin prepared for electron microscopy by the Ficoll-drying procedure displays a discontinuous fiber; within this fiber, nucleosomes are clustered in knobs or superbeads. Contrary to this arrangement, Finch and Klug (4) observed that chromatin fragments of up to 40 nucleosomes in length, generated by brief micrococcal nuclease treatment of rat liver chromatin, appear as a supercoid or solenoid 250–300 Å in diameter in the presence of low concentrations of Mg^{++}. The helical nature of the chromatin fiber was also noted by Filip et al. (3) in high-voltage electron micrographs of water-spread, acute-angle shadowed chromosome preparations and by Ris and Korenberg (15) in high-voltage electron micrographs of chromatin prepared in the presence of mono- and divalent cations.

Until recently, however, it has not been possible to visualize simultaneously discrete nucleosomal subunits and intact higher-order chromatin fibers (10, 12, 13). We recently reported that mitotic cells, disrupted by agitation with small glass beads in the absence of nonionic detergent and formalin, release chromosomes in which 200- to 300-Å diameter higher-order chromatin fibers are minimally disrupted (13). Negative staining of such samples prepared for electron microscopy by the Miller technique (7) made it possible to observe individual nucleosomes within these fibers which are arranged in several distinct packing patterns. Thus, although the higher-order fiber is of uniform diameter, the different packing patterns visualized are not consistent with a single mode of folding of the 100-Å fiber to form this higher-order structure. In addition, chromosomes prepared in the presence of nonionic detergent, piperazine-*N*-*N*′-bis(2-ethane sulfonic acid) (PIPES) buffer, $MgCl_2$, and EGTA provided preparations in which it was possible to observe the unfolding of these fibers into irregular nucleosome aggregates (superbeads), closely packed nucleosomes within 100-Å fiber and, finally, beads-on-a-string (14). In this report, we present observations on the morphology of nucleosome packing patterns within higher-order chromatin fibers of partially

disrupted interphase nuclei from mouse L929 cells. These data are discussed in view of nucleosome organization within these fibers.

MATERIALS AND METHODS

Mouse L929 cells from the American Type Tissue Collection (Rockville, Md.) were grown in monolayer cultures in Joklik's suspension medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum (Irvine Scientific). Metaphase cells were removed from logarithmically growing cultures by selective detachment, and the remaining interphase cells were mechanically removed from the surface of the flask with a rubber policeman, pelleted, washed, and resuspended in unsupplemented Joklik's suspension medium. For most preparations, an equal volume of 0.5-mm glass beads (B. Braun Instruments, San Francisco, Calif.) was added to an aliquot of cells in a conical glass centrifuge tube, and cells were lysed by vortex-mixing for 5 s at maximum speed. This treatment resulted in the lysis of 10–20% of the cells with the release of partially ruptured nuclei. Alternatively, cells and nuclei were lysed by dilution with H_2O (pH 9.5). The lysates were prepared for electron microscopy by the Miller procedure as detailed in Miller and Bakken (6) except that formalin was omitted from the cushion through which samples were centrifuged because its presence promotes fiber dispersal (11). Grids were negatively stained with 0.005 M uranyl acetate in methanol as described previously (13).

RESULTS AND DISCUSSION

When interphase nuclei are subjected to nonionic detergent treatment, the nuclear membrane lyses and chromatin fibers are released which display a beads-on-a-string morphology (8). Analogous structures are observed after hypotonic lysis if samples are exposed to 10% formalin (11). This relaxation of normally highly folded chromatin fibers is prevented if samples are not exposed to formalin and/or detergent. Thus, chromatin obtained from cells lysed either by mechanical disruption in the presence of small glass beads or by hypotonic shock and prepared for electron microscopy in the absence of formalin retains higher-order structure. Electron microscopy of such material shows loops of chromatin extending from the margin of an otherwise highly compacted nuclear mass (Fig. 1). Further release of chromatin fibers from the nucleus results in extensive arrays of higher-order fibers spread across the grid; in such samples, one usually does not visualize the remainder of the nucleus. The loops emanating from lysed nuclei are, for the most part, uniform in diameter (200–300 Å) and, in negatively stained preparations, they appear as a continuous array of closely apposed nucleosomes (Figs. 2–5). The distribution of nucleosomes along the length of the fiber does not appear uniform; a variety of packing patterns can be identified which reflect different views of the higher-order fiber either very similar to its native state or somewhat distorted as a result of the preparative procedures (see reference 10).

A description of the prevalent packing patterns provides some information on the organization of the higher-order fiber. Two or three nucleosomes span the width along a major portion of the fiber (Fig. 2), and adjacent nucleosomes are packed along the fiber length in either a staggered or a stacked conformation (Figs. 3–5). One frequently sees regions which appear knobby or ropelike (Fig. 3); in these regions, nucleosomes are arranged in a staggered conformation. This appearance is accentuated where the fiber contains a single nucleosome separating long stretches of closely apposed nucleosomes (Fig. 3); such fiber morphology is infrequent and may reflect a transition from one packing pattern to another. This knobby morphology is consistent with the conformation of the 250-Å fiber as described by several workers using a variety of preparative procedures (for review, see reference 14). Occasionally, bands of nucleosomes can be seen passing diagonally across the fiber (Figs. 4 and 5); where several rows are seen, the fiber often appears as a twisted ribbon (Fig. 4). Another prevalent pattern exhibits two parallel rows of nucleosomes in which the individual subunits appear to be stacked along the fiber length (Fig. 2). Although this conformation can be interpreted to represent two nucleosome-containing fibers side-by-side, observations on the sequential unfolding of 250-Å fibers provide evidence in support of the hypothesis that this fiber is, in fact, formed by compaction of a single 100-Å fiber (14).

Our observations that nonionic detergent treatment and/or exposure of chromatin to buffered 10% formalin induce rapid unfolding of the uniform diameter higher-order fiber may provide an explanation for other workers' inability to observe such fibers in material obtained from detergent-lysed samples or prepared by the standard Miller procedure (6) which involves centrifugation of the sample through 10% formalin. These two agents appear to disrupt the 200- to 300-Å fiber in different ways because a detergent-lysed sample in medium or PIPES-Mg^{++}-EDTA prepared im-

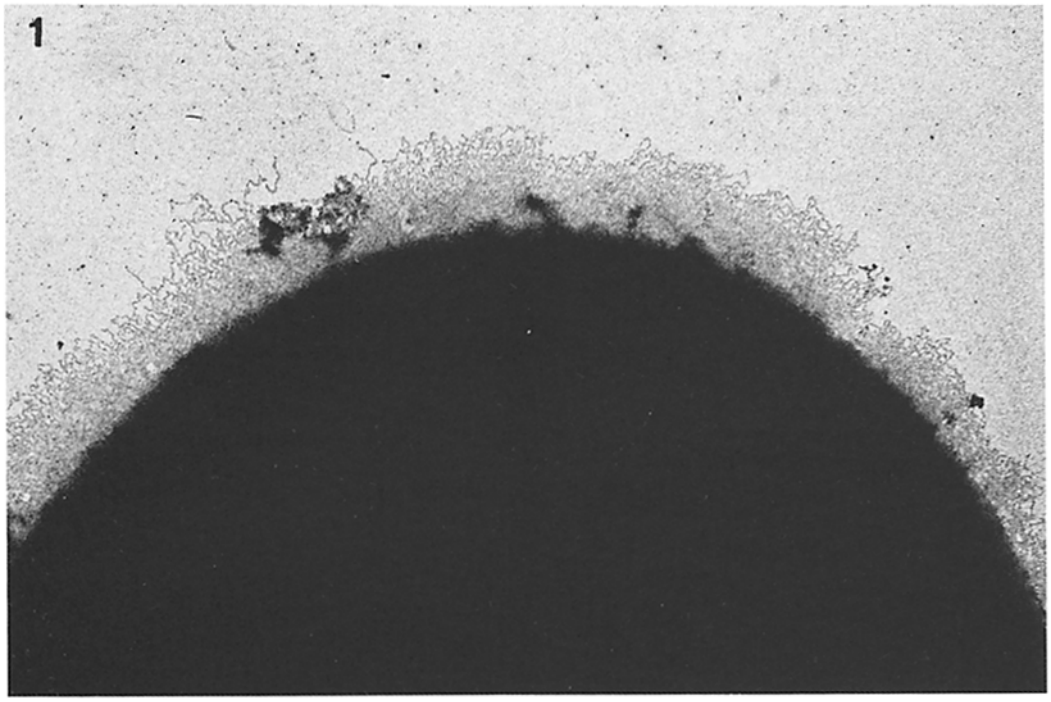
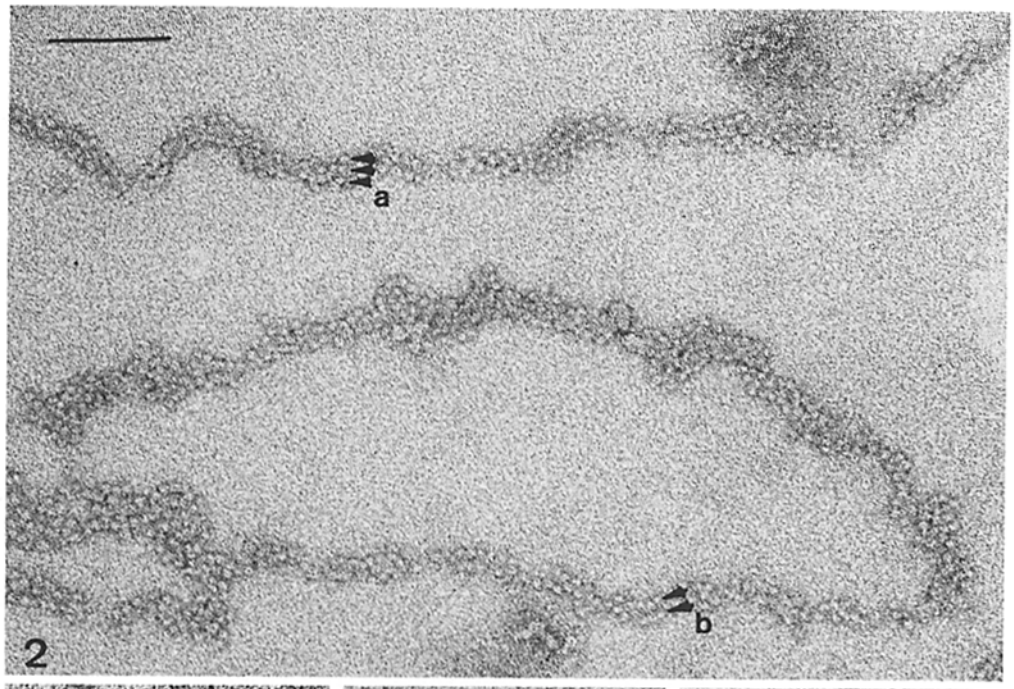


FIGURE 1 Low magnification electron micrograph of interphase nucleus from a mouse L929 cell. Numerous higher-order fibers extend as loops from the ruptured nuclear envelope. Bar, 1.0 μm. × 8,000.

mediately after cell lysis in the absence of formalin exhibits higher-order fibers of uniform diameter; with time, however, these fibers break down (14). Contrary to this instability, higher-order fibers obtained from either mechanically lysed or hypotonically shocked nuclei are stable for at least 1 h at room temperature in the absence of formalin. Treatment of such higher-order fiber-containing samples with 10% formalin induces rapid, and possibly instantaneous, unfolding. Because H1 is extracted from chromatin at ≥1% formaldehyde (3.7% formalin) and pH's >8.2, it is likely that the formalin effect is related to H1 removal. We are now performing experiments to document this.

The structure of the higher-order fiber visualized in our preparations emphasizes the fact that individual nucleosomes remain as identifiable units even within tightly packed arrays as argued by Olins (10). Our preparations, however, do not support the model proposed by Hozier et al. (5) that the fiber is composed of a series of discrete superbeads, nor the model of Finch and Klug (4) that the fiber is a uniform solenoid. The superbead configuration is prominent in both metaphase and interphase chromatin when the higher-order fiber is unwinding to form the 100-Å fiber, thus suggesting that it may represent a metastable intermediate in chromatin organization (14). Although we find no evidence for extensive helical coiling within the 200- to 300-Å fiber, certain packing patterns are consistent with relatively short regions of solenoid (Figs. 3 and 5).

The variety of nucleosomal packing conformations and their variable distribution along the length of the fiber suggest that the basic 100-Å fiber is not uniformly packed within the 200- to 300-Å fiber. Such variability may reflect compositional differences in nucleosomes which are related to the function of the DNA sequences associated with these nucleosomes. For example, the presence of modified histones and/or nonuniformly distributed nonhistone chromosomal proteins could alter the number of nucleosomes per turn of a solenoid as suggested by Worcel (16). Alternatively, this variability may be essential for the further folding of the 200- to 300-Å fiber. These data and those derived from metaphase chromosome preparations illustrate that, under the same conditions, the 200- to 300-Å fiber class exists as an equally stable structure regardless of the degree of chromatin condensation in the cell.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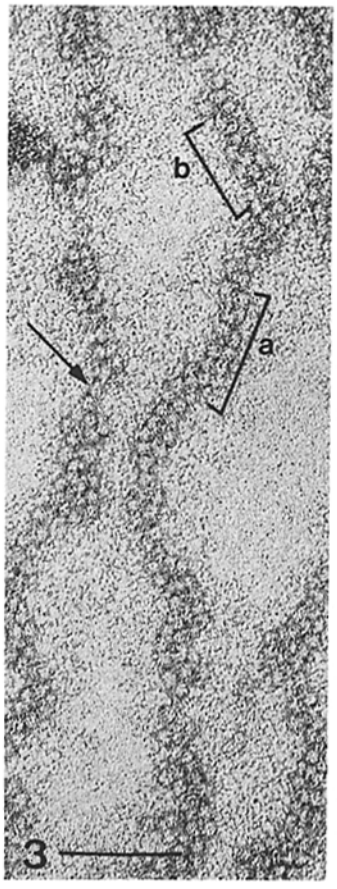
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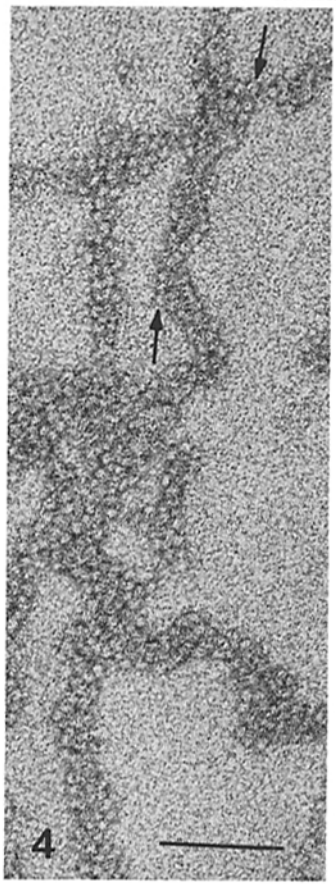
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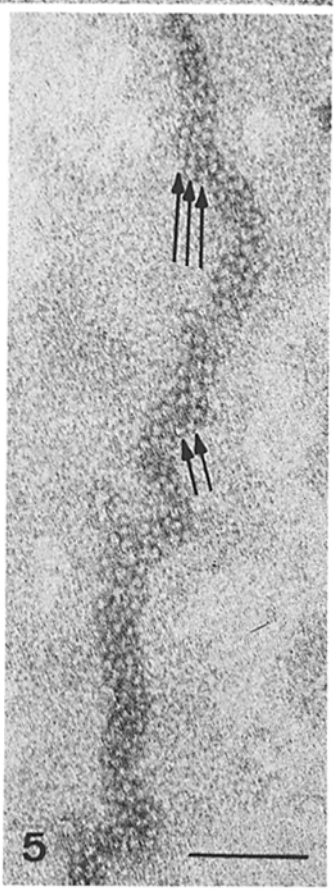
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An obvious extension of this work is the elucidation of higher-order structure along chromatin segments active in RNA synthesis and DNA replication. The system used in the present studies is not optimized for such analyses because the level of RNA synthesis at any one site is, on the average, very low and the vast majority of cells are not in S phase. We are pursuing these problems in more suitable systems. In addition to these studies, we would like to provide a three-dimensional model for the manner in which the 100-Å fiber is folded. Because the available micrographs display only occasional short straight regions, they are unsuitable for optical diffraction studies. We are developing modifications in preparative procedures which may provide samples that are better suited for image analysis.

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Figure 2 Loops of interphase chromatin from mouse L929 cells formed by closely apposed arrays of nucleosomes. 2–3 nucleosomes span the width of the fiber (*a*, *b*). Bar, 0.1 μm. × 100,000.

Figure 3 Chromatin fibers displaying nucleosomes in a stacked (*a*) or staggered (*b*) conformation. Regions containing a single nucleosome (arrow) may represent transitions between different packing arrangements (arrow). Bar, 0.1 μm. × 170,000.

Figure 4 Chromatin fiber in which a band of nucleosomes (arrow) imparts a twisted ribbon appearance to the fiber. Bar, 0.1 μm. × 160,000.

Figure 5 Chromatin fiber displaying prominent bands of nucleosomes (arrow) which transect the fiber. Bar, 0.1 μm. × 110,000.