Ultrastructural Organization of Yeast Chromatin

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ABSTRACT The ultrastructural organization of yeast chromatin was examined in Miller spread preparations of samples prepared from spheroplasts or isolated nuclei of *Saccharomyces cerevisiae*. Micrographs from preparations dispersed in 1 mM Tris (pH 7.2) illustrate that the basic chromatin fiber in yeast exists in two ultrastructurally distinct conformations. The majority (up to 95%) of the chromatin displays a beaded nucleosomal organization, although adjacent nucleosomes are separated by internucleosomal linkers of variable lengths. Ribonucleoprotein (RNP) fibrils are only occasionally associated with chromatin displaying the conformation. The remaining 5–10% of the chromatin appears to be devoid of discrete nucleosomes and has a smooth contour with a fiber diameter of 30–40 Å. Transcriptional units, including putative ribosomal precursor RNA genes, defined by the presence of nascent RNP fibrils are restricted to chromatin displaying this smooth morphology. Chromatin released from nuclei in the presence of 5 mM Mg^{++} displays higher-order chromatin fibers, 200–300 Å in diameter; these fibers appear to be arranged in a manner that reflects the two forms of the basic chromatin fiber.

The alternating arrangement of nucleosomes and internucleosomal DNA typical of eukaryotic chromatin imparts a regular beaded morphology to the basic chromatin fiber when spread preparations are viewed in the electron microscope (34). *In vivo* this fiber is folded to form a higher-order chromatin fiber, 200–300 Å in diameter, that is present in both interphase nuclei and metaphase chromosomes (for review, see reference 36). Although the exact manner in which the nucleosomes are arranged within this fiber remains controversial (10, 18, 22, 36, 38, 43, 46), histone H1 has been implicated in the formation and/or maintenance of this fiber class (3, 5, 10, 24, 43).

Several lines of investigation have addressed the question of how this basic chromatin organization is modified to accommodate differential gene expression. Biochemically, transcribed chromatin appears to be distinct from nontranscribed chromatin in conformation. Active regions typically are preferentially digested by DNase I and micrococcal nuclease (2, 4, 15, 42, 44); in some cases they are preferentially associated with HMGs 14 and 17 (45); and there are some reports that these regions are depleted of H1 (24, 25). Ultrastructurally, segments of chromatin that display low levels of transcriptional activity appear to be indistinguishable from inactive beaded chromatin (7, 8, 12, 23, 30). However, there is a trend towards a lower density of nucleosomes per length of chromatin as transcriptional activity increases. Reports of smooth chromatin typically are confined to ribosomal chromatin or nonribosomal chromatin that exhibits high levels of transcriptional activity (13, 14, 30, 31, 39). In two cases, however, nonbeaded chromatin does not appear to be confined to regions of high transcriptional activity. In *Oncopeltus fasciatus* embryos, there is a transition from a beaded to a nonbeaded morphology of ribosomal gene chromatin that is detected just before activation of RNA synthesis (11). In addition, 10–20% of the nonribosomal chromatin in *Physarum polycephalum* interphase nuclei appears to be smooth, regardless of the level of transcriptional activity (40).

In contrast to most eukaryotes, the yeast genome possesses an unusually high proportion of transcribed chromatin. Approximately 20% of yeast single copy DNA is complementary to mRNA, suggesting that at least 40% of the genome is transcribed (17). Despite this, all yeast chromatin appears to be equally susceptible to DNase I, and these data have been used to suggest that the entire genome exists in the same conformational state (26). In addition, a typical H1 protein has not been identified in yeast (6). Although protein with similar function may exist, the absence of H1 suggests that yeast chromatin may possess a distinct higher-order structure. In light of these interesting structural properties of yeast chromatin, we undertook an ultrastructural analysis of yeast chromatin. Micrographs from this study illustrate that the basic chromatin fiber in yeast exists in two morphologically distinct conformations and that a proportion of the genome exists in an extended and, perhaps, potentially active state for long periods during the cell cycle. The basic chromatin fiber appears to be capable of forming supranucleosomal structures whose organization may reflect the heterogeneity of this fiber.

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MATERIALS AND METHODS

Saccharomyces cerevisiae chromatin was prepared from logarithmically growing cultures of strains SKQ-2N and 20B-12α grown in YEPD medium at 30°C. Spheroplasts were produced by incubating cells, washed in distilled water for 5–7 min, in YEPD media containing 1 M sorbitol, 1% mercaptoethanol, and Zymolyase (5 mg/ml). In some preparations sodium butyrate (30 mM), actinomycin D (100 μg/ml), or cyclohexamide (100 μg/ml) was included in the spheroplasting solution. Spheroplasts or samples of freshly isolated nuclei, prepared by the procedure of Ide and Saunders (19), were diluted 1:20 with 1 mM Tris (pH 7.2) and centrifuged through a 1 M sucrose cushion containing 10% formalin (pH 9.0) onto electron microscope grids according to the procedure of Miller and Beatty (32). For preparations of higher-order fibers, spheroplasts were mechanically lysed with 1-mm glass beads (35) in the presence of 5 mM Mg^{++}. Mouse L929 cells were cultured and prepared as previously described (33). Samples were centrifuged through a cushion of 1.0 M sucrose (pH 7.2) containing 5 mM Mg^{++}. All samples were dried out with Photoflo and shadowed using Pt:Pd wire (80:40) before examination in a Siemens 1A electron microscope operated at 60 kV.

RESULTS

Micrographs of spread preparations of yeast chromatin obtained from spheroplasts or isolated nuclei of *Saccharomyces cerevisiae* reveal a beaded morphology along 90–95% of the chromatin fibers (Fig. 1 *a* and *b*). In contrast to the ultrastructural arrangement of most eukaryotes, adjacent nucleosomes in yeast are separated by internucleosomal linkers of variable lengths. Measurements of linker lengths in micrographs taken from several different preparations indicate that there is an approximately equal distribution of lengths between 10 and 500 Å. We were unable to detect a patterning of size classes along the length of the chromatin fiber. The remaining 5–10% of the chromatin appears devoid of discrete nucleosomes and has a smooth contour with a diameter in unshadowed preparations of 30–40 Å. These dimensions suggest that nonbeaded chromatin fibers are not naked DNA but that the DNA is associated with protein. The transition between a beaded and nonbeaded morphology occurs quite abruptly in most regions of the fiber (Fig. 2). Smooth chromatin was not confined to a particular portion of the dispersed chromatin but rather appears to be intermingled with beaded chromatin. Thus, yeast chromatin exhibits two ultrastructurally distinct morphologies with a portion of the genome existing in an extended conformation. This extended conformation may be more transitory and therefore undetected in specimens from higher eukaryotes.

We have performed several experiments to investigate the possibility that the different conformations described above are generated during sample preparation. It has been demonstrated that yeast chromatin, which is highly acetylated in vivo, can undergo rapid deacetylation during experimental manipulation (9). We therefore prepared samples of yeast chromatin for electron microscopy in the presence of 30 mM sodium butyrate in order to maintain in vivo levels of histone acetylation. Micrographs from these preparations revealed chromatin fiber with a morphology identical to that illustrated in Fig. 1. Although it is clear that hyperacetylated chromatin has a different conformation as assayed by DNase I sensitivity (33), our experiment argues that the ultrastructural differences observed are independent of the acetylation state of the chromatin.

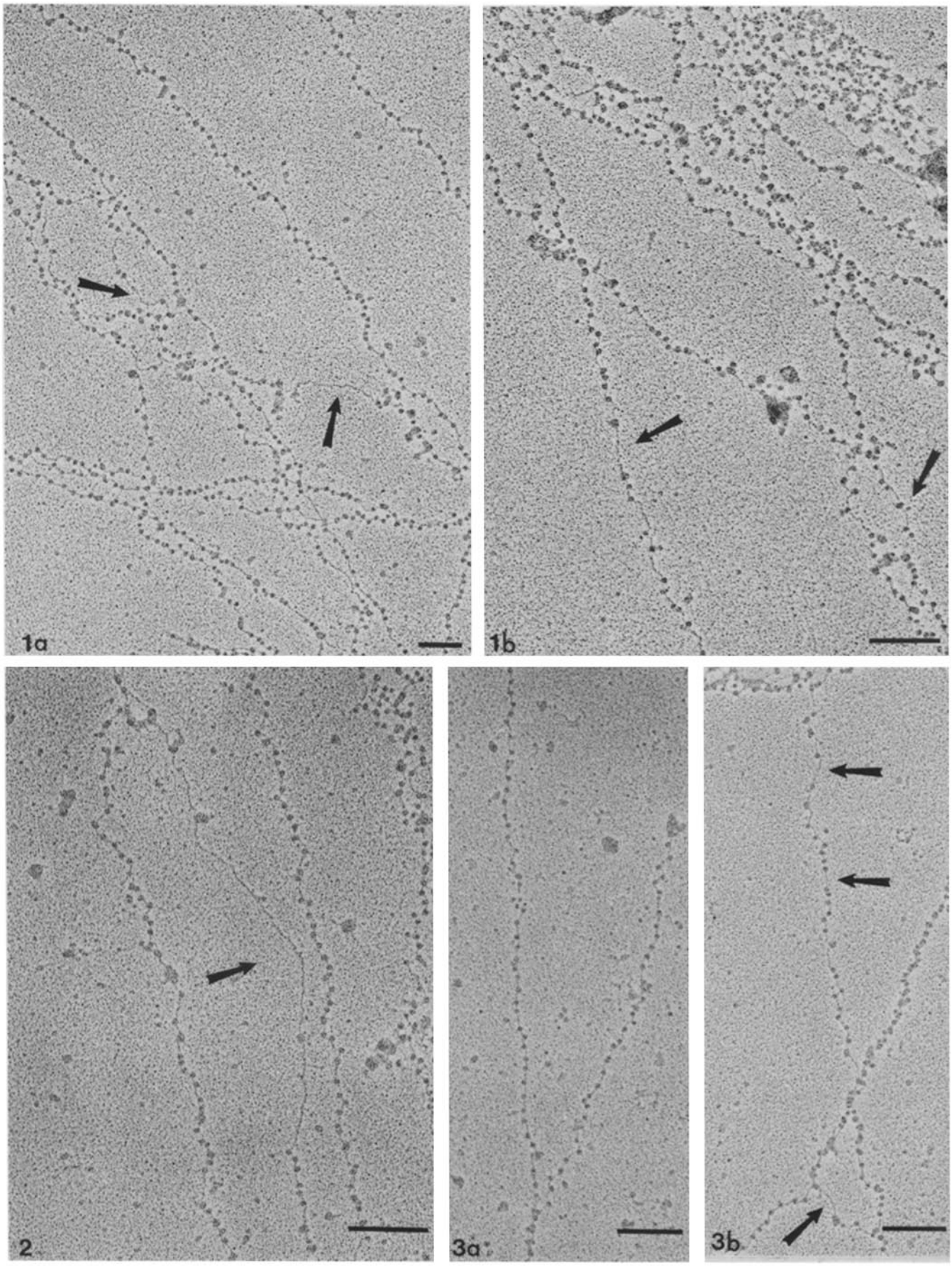
We then investigated the possibility that there are proteases or other substances in the spheroplasting solution that might induce structural alterations in DNA-histone interactions along the chromatin fiber. We included mouse L929 cells in the spheroplasting solution along with the yeast cells since spread mouse chromatin possesses a generally regularly spaced organization of nucleosomes and the absence of long regions of smooth chromatin (35). When these cells are co-spread and examined in the electron microscope, chromatin extending from the margin of the large partially lysed mouse nuclei displayed its typical regular beaded organization (Fig. 3 *a*) while chromatin extending from the smaller yeast nuclei displays a heterogeneous morphology (Fig. 3 *b*). Although there is some variability in internucleosomal DNA lengths inherent in all Miller spread preparations of chromatin, the variations observed in Fig. 3 *a* do not approach those visualized in Fig. 3 *b*. The fact that mouse chromatin does not acquire greater heterogeneity in structure as a consequence of being co-spread with yeast argues against the existence of a trans-acting substance in the spheroplasting solution which is responsible for the variations in the ultrastructural morphology of the yeast chromatin fiber. In some organisms, nucleosomes are lost along portions of the chromatin fiber when certain detergents are used to disperse the nuclear contents before examination in the electron microscope (Rattner, unpublished data; 13). Yeast chromatin dispersed in distilled water (pH 9.5), 1% Nonidet P-40 (pH 9.0), 0.01% Joy (Proctor and Gamble, Cincinnati, OH) (pH 7.0), or obtained by mechanical cell lysis with glass beads and centrifuged through 10% formalin (pH 9.0), revealed the same chromatin morphologies described above. These experiments suggest that the two ultrastructurally distinct conformations described are a reflection of in vivo structure and are not the result of gross structural rearrangements induced during specimen preparation. Nascent RNP fibrils were most readily detected in samples prepared in the presence of either actinomycin D or cyclohexamide. We presume that this is due to inhibition of run-off transcription that apparently occurs during preparation. When these drugs were included in the spheroplasting solution, micrographs were obtained that showed abundant ribosomal and nonribosomal transcriptional activity (Fig. 4 *a–c*). The putative ribosomal precursor RNA transcripts illustrated in Fig. 4 *b* display terminal knobs characteristic of those reported for the rRNP fibrils of variety of eukaryotes (30). All the ribosomal transcription units present within a particular nuclear spread do not appear to be equally active. This may reflect modulation in their transcription, or perturbation in activity induced during specimen preparation.

The large number of nascent RNP fibrils in these specimens probably reflects the high level of transcriptional activity char-

FIGURE 1 (*a* and *b*) Yeast chromatin dispersed from (*a*) spheroplasts and (*b*) isolated nuclei. Arrows indicate linkers of variable lengths and nucleosome-free chromatin. Bar, 0.1 μm.

FIGURE 2 Nonbeaded region of a yeast chromatin fiber dispersed in 1 mM Tris (pH 7.2). Note the discrete transition between the beaded and nonbeaded portions of the fiber. Bar, 0.1 μm.

FIGURE 3 Co-spread mouse and yeast chromatin; *a* illustrates the regular beaded arrangement of mouse chromatin, and *b* illustrates the heterogeneous appearance of yeast chromatin. Arrows in *b* indicate linkers of variable length. Bar, 0.1 μm.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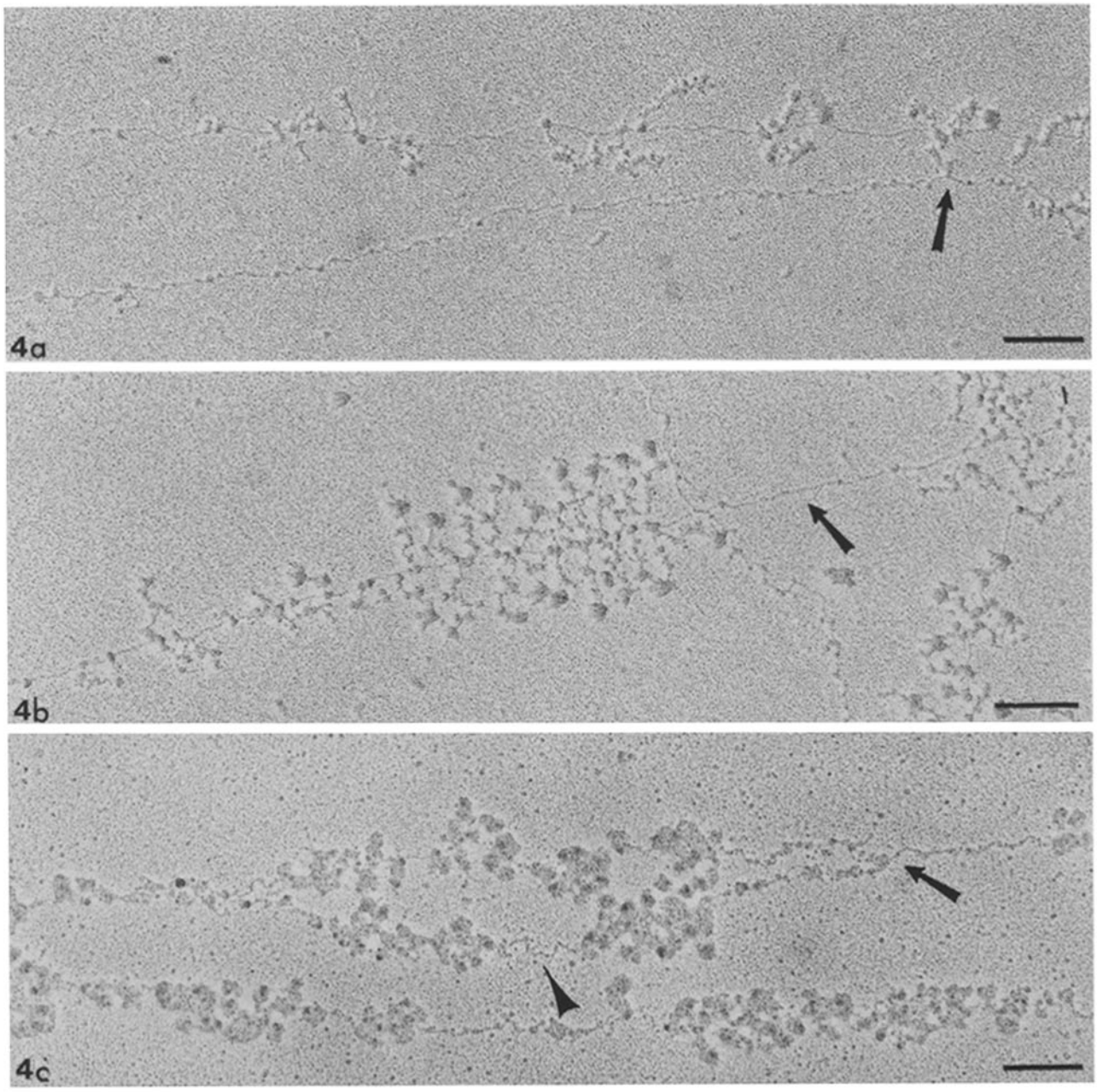

FIGURE 4 Active transcription units. (*a*) Nonribosomal transcription unit displaying a high level of transcriptional activity and a nonbeaded morphology between nascent RNP fibrils. Arrow indicates a single transcript along a region of the fiber showing a beaded morphology. (*b*) Ribosomal transcription unit: the nonbeaded morphology extends beyond the region associated with nascent RNP fibrils. (*c*) Putative replication fork (arrow). Sister chromatids display a high level of transcriptional activity. Arrowhead indicates nucleosome-free region between RNPs. Bars, 0.1 μm.

acteristic of yeast chromatin throughout the cell cycle (16). In general, our preparations illustrate that both ribosomal (Fig. 4*b*) and nonribosomal (Fig. 4*a* and *c*) transcription units that contain a high density of RNP fibrils tend to be associated with those regions of the chromatin fiber that exhibit a smooth, nonbeaded morphology. Fig. 4*c* shows a putative replication fork that displays a high level of transcriptional activity along both strands within the fork. In this case, nonbeaded chromatin is confined to the region of the fiber associated with nascent RNPs. Occasionally, however, the nonbeaded conformation extends beyond the region directly associated with RNP fibrils, suggesting that this organization is not confined to actively transcribed sequences. This supposition is further supported by the absence of nascent RNP fibrils along a majority of the nonbeaded chromatin visualized in our spread preparations. Single RNP fibrils are scattered throughout these preparations and are associated with beaded chromatin fibers (Fig. 4*a*). These transcripts probably reflect a small proportion of the total transcriptional activity in the nucleus. Nonetheless, in yeast, as in many other eukaryotes, there is a tendency towards a reduction in the number of nucleosomes/unit length of chromatin as the frequency of nascent RNP fibrils increases.

In order to detect the possible existence of supranucleosomal organization within the yeast nucleus, chromatin was released from spheroplasts by mechanical lysis in the presence of 5 mM Mg^{++}. Micrographs from these preparations (Fig. 5 *a*) illustrate

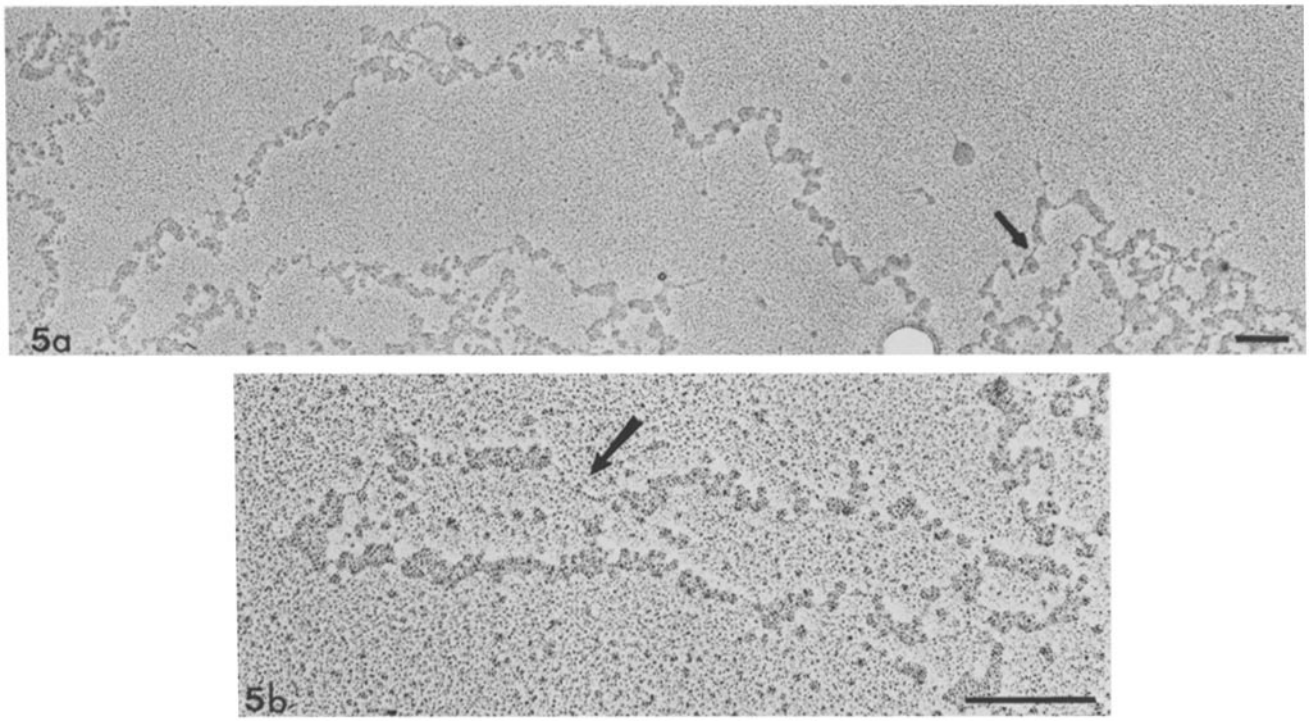

FIGURE 5 (*a* and *b*) Higher-order chromatin fibers obtained from mechanically lysed spheroplasts in the presence of 5 mM Mg^{++}. Arrows indicate nonbeaded chromatin separating regions displaying supranucleosomal organization. Bar, 0.1 μm.

higher-order chromatin fibers that appear to be composed of supranucleosomal clusters. These higher-order structures frequently appear as oblong units or irregular clusters that vary in length but generally have a diameter of 200–300 Å. In addition, regions of the higher-order fiber occasionally are separated by chromatin stretches that are devoid of nucleosomes (Fig. 5 *a* and *b*). These regions probably correspond to the larger linkers and/or nonbeaded chromatin detected in more dispersed preparations. Therefore, despite the apparent lack of a traditional H1 histone, yeast chromatin can be organized into higher-order fibers that are somewhat similar to the equivalent fiber class in higher eukaryotes.

DISCUSSION

This study illustrates that yeast chromatin exists in two ultrastructurally distinct forms. The beaded chromatin conformation represents 90–95% of the chromatin visualized in our preparations. It differs from the morphology of chromatin in higher eukaryotes in that internucleosomal linker regions appear to vary in length. This variation may be an intrinsic characteristic of yeast chromatin, or it may represent a structural alteration induced during specimen preparation. Nuclease digestion studies suggest that yeast chromatin possesses a 165 base pair repeat (28) although there is some evidence for heterogeneity (25, 27, 29). Since micrographs from co-spread mouse and yeast nuclei show that each maintains individual ultrastructural morphology under our preparative conditions, there is no evidence for the existence of a trans-acting substance inducing gross structural alterations. A major biochemical difference in the chromatin of these two organisms is the apparent lack of histone H1 in yeast (6). It is possible that the absence of this histone, which may function to clamp together DNA folded around the nucleosome (41), may render nucleosomes more mobile during manipulation; this could result in unfolding of variable amounts of core DNA from the nucleosome, causing an increase in linker lengths. However, the studies of Thoma and co-workers (43) suggest that one would not expect a large number of large linker lengths to be generated in this way under the conditions employed in this study. Therefore, some but not all of the variability in linker length could be due to unfolding of core DNA.

Chromatin free of discrete nucleosomes is present in 5–10% of the material visualized in our preparations. It is important to note that this smooth chromatin is not naked DNA but, based on its diameter and staining, is associated with other material, presumably proteins. Although the nature of the DNA-containing complex is unknown, it may represent nucleosomes that are in an unfolded conformation in vivo or nucleosomes that are more labile and, therefore, are selectively unfolded during preparation. These regions may be the ultrastructural analogue of the so-called A particles described in *Physarum* ribosomal chromatin by Johnson et al. (21). These structures are identified as a subclass of monomer nucleosomes that unfold during sedimentation in sucrose gradients. In this system, the coding regions but not intergenic spacers are enriched in the slower sedimenting fraction (20).

Measurements obtained from synchronized cell samples derived from nine elutriator fractions suggest that the proportion of nucleosome-free chromatin remains relatively constant throughout the cell cycle (37). As described above for a logarithmic culture, a small proportion of these nucleosome-free regions displays a high density of nascent RNP fibrils and, in some cases, the smooth contour of the fiber extends beyond the confines of the region displaying active transcription. Thus, a portion of the yeast genome appears to exist in an extended state throughout the cell cycle, and this conformation is not dependent upon the association or density of RNP fibrils. Nonbeaded chromatin fibers in yeast may represent sites of high transcriptional activity that are periodically expressed at discrete points during the cell cycle. The activation of these regions may be sufficient to stably alter chromatin structure

such that histone-DNA contacts are maintained in a more labile fashion. If such regions were maintained between periods of transcription, they might be expected to be visualized as smooth fibers. This extended but nontranscribed state may be analogous to the ribosomal chromatin of *O. fasciatus* described by Foe and co-workers (11, 12). It is therefore possible that the same regions exist as smooth chromatin throughout the cell cycle. Our micrographs illustrate that the basic chromatin fiber in yeast is folded into a heterogeneous higher-order fiber. This heterogeneity may be a reflection of variable linker lengths, large nonbeaded regions that do not participate in supranucleosomal organization, and/or the absence of histone H1. Most models for higher-order chromatin organization suggest a fundamental role for histone H1 in the formation and/or maintenance of higher-order folding (1, 36). Therefore, in yeast, histone H1 may be replaced by an as yet unidentified molecule.

The relationship between gene expression and the organization of higher order fibers, beaded chromatin regions of variable linker length, and smooth chromatin fibers is still unclear. However, the ultrastructural analysis of plasmid-borne genes in yeast during transcriptional activation presents a promising system in which to address these problems.

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