Nucleosomes in metaphase chromosomes

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Received 24 February 1976

ABSTRACT

Previous studies of the structure of metaphase chromosomes have relied heavily on electron micrography and have revealed the existence of a 10-nm unit fiber that is thought to generate the native 23-30-nm fiber by higher order folding. The structural relationship of these metaphase fibers to the interphase fiber remains obscure. Recent studies on the digestion of interphase chromatin have revealed the existence of a regularly repeating subunit of DNA and histone, the nucleosome that generates the appearance of 10-nm beads connected by a short fiber of DNA seen on electron micrographs. It was therefore of interest to probe the structure of the metaphase chromosome for the presence of nucleosomal subunits. To this end metaphase chromosomes were prepared from colchicine-arrested cultures of mouse L-cells and were subjected to digestion with staphylococcal nuclease. Comparison of the early and limit digestion products of metaphase chromosomes with those obtained from interphase nuclei indicates that although significant morphologic changes occur within the chromatin fiber during mitosis, the basic subunit structure of the chromatin fiber is retained by the mitotic chromosome.

INTRODUCTION

The DNA of the metaphase chromosome is compacted into a series of hierarchical structures, presumably through association with the chromosomal proteins. Analysis of the organization of DNA within the mitotic chromosome has relied heavily upon electron microscopic observations. These studies have revealed a 10 nm unit fiber, which by specific folding is thought to generate the native fiber, 23-30 nm in thickness (1, 2). It is generally assumed that the unit fiber of interphase chromatin is maintained during the process of condensation that results in the formation of the metaphase chromosome. Support for this concept could be provided if it could be demonstrated that the structural features of the interphase fiber are retained in preparations of metaphase chromosomes. One property of the unit fiber of interphase chromatin is the presence of regularly repeating subunits or nucleosomes containing from 180-200 base pairs of DNA and 8-10 histone molecules (3-8). This organization generates a microscopic picture
of contiguous 10-nm beads connected by a short fiber of DNA (9-10) and is characteristic of the interphase nucleus of virtually all eucaryotes examined. The appearance of repeated 7-9-nm beads has recently been reported in preparations of metaphase chromosomes from mouse L-cells (11). In this report we confirm by nuclease digestion that the basic subunit structure of interphase chromatin is retained in the mitotic chromosome, and suggest that the organization of DNA into nucleosomes is responsible for the 10 nm unit fiber observed in the metaphase chromosome.

METHODS

Preparation of Metaphase Chromosomes and Interphase Nuclei

Both nuclei and chromosomes were isolated by a modification of the procedure of Willecke and Ruddle (12). Mouse L-cells were grown as monolayer cultures. Colchicine (0.5 μg/ml) was added to cells in logarithmic growth. After 12-18 hrs, cells in metaphase arrest were harvested by selective detachment from the culture dish by gentle shaking. To obtain interphase cells as controls, colchicine-treated cultures were shaken vigorously to remove metaphase cells and washed repeatedly with phosphate-buffered saline (PBS). Interphase cells were scraped into PBS. Cells were washed several times in phosphate-buffered saline, suspended in 1 ml of 15 mM Tris-HCl, pH 7.0, 3 mM CaCl₂, 0.2% Triton-X-100, and disrupted in a Dounce homogenizer. The homogenate was centrifuged at 4000 X g and the pellet was suspended in the above buffer and again centrifuged. This procedure was performed twice in the presence of Triton and repeated in the absence of Triton. Nuclei or chromosomes were then resuspended in 1 mM Tris-HCl, pH 7.9, 0.1 mM CaCl₂ at a DNA concentration of 250 μg/ml.

Nuclease Digestion of Chromosomes and Nuclei

Staphylococcal nuclease (1 μg/ml) (Worthington Biochemical Corp.) was added to suspensions of nuclei or chromosomes and at various times aliquots were removed. The extent of digestion was determined by the solubility of A260-absorbing materials in 1MHC104-1M NaCl. Nuclease reactions were terminated by the addition of NaEDTA to 5 mM. Resistant DNA was purified by phenol extraction as previously described (6).

Gel Electrophoresis

Slab gel electrophoresis of purified DNA digestion products was performed in either 2.5% acrylamide–0.5% agarose or 6% acrylamide as previously described (6). For gel electrophoresis of nucleoprotein com-
plexes, the gel buffer contained 0.04 M Tris-HCl, pH 7.9, 0.005 M sodium acetate and 0.001 M EDTA. Two microliters of 40% sucrose--1% Bromophenol Blue were added to 10 μl containing 10 μg of DNA, and the samples were run at 200 V on a 17 cm cooled slab gel for 2 hrs. Gels were stored for 30 min in ethidium bromide (1 μg/ml) and photographed under UV light.

RESULTS

Two approaches have been used to demonstrate the presence of nucleosomes in animal cells: a) direct visualization by electron microscopy and b) biochemical isolations following mild nuclease digestion of chromatin. We have chosen to disrupt mitotic cells in Triton-containing buffers of low ionic strength. Chromosomes prepared in this way maintain a highly condensed structure and retain a number of ultrastructural features of intact chromosomes (11, 12). These preparations of mitotic chromosomes therefore appeared to be valid substrates for structural studies using the enzyme staphylococcal nuclease. Chromosomes were obtained from colchicine-arrested cells after selective detachment from the culture plate by gentle shaking. Control interphase nuclei were isolated from those cells that remained adherent to the culture dish. When stained and examined by light microscopy, metaphase cells prepared in this manner were contaminated with less than 10% interphase cells. Similarly, interphase cells were greater than 90% pure. Prior to nuclease treatment, chromosomes were examined by light microscopy and although fragmented were found to retain their condensed structure.

Digestion of either metaphase chromosomes or intact nuclei results in the solubilization of about 50% of the chromatin DNA. Of interest is the observation that the kinetics of digestion of mitotic chromosomes are virtually identical to those observed with intact nuclei (data not shown). Furthermore, enzymatic digestion of interphase nuclei prepared from cells incubated in the presence or absence of colchicine reveals that the drug has no discernible effect upon the kinetics of nuclease attack. It appears that although the DNA of the metaphase chromosome is far more compact than in interphase cells, those sites sensitive to nuclease attack are equally accessible to enzyme in the two cell phases.

Examination of the DNA fragments generated during the course of the digestion of interphase nuclei has revealed two levels of structure within the chromatin complex: the organization of nucleosomes themselves and
the arrangement of DNA within the individual nucleosomes. It was, therefore, of interest to examine the products of nuclease digestion of the metaphase chromosomes. To this end, preparations of mitotic and interphase chromosomes were subjected to increasing levels of digestion with the enzyme staphylococcal nuclease. The resulting nucleoprotein complexes were freed of protein and analyzed by electrophoresis in 2.5% acrylamide--0.5% agarose gels. In Fig. 1 we observe at the earliest times in the diges-

![Figure 1. Polyacrylamide gel electrophoresis of purified DNA fragments liberated upon digestion of metaphase chromosomes and interphase nuclei. Nuclei and chromosomes were prepared as described in Methods and digested with staphylococcal nuclease. At various times, aliquots were removed and DNA purified. 10 µg of DNA were applied to each slot of a 2.5% acrylamide--0.5% agarose slab, electrophoresed at 200 V for 2 hrs. Gels were stained with ethidium bromide and photographed. Samples from right to left represent 2, 4 and 10% digestion of metaphase chromosomes and 2, 4, and 10% digestion of interphase nuclei.](https://academic.oup.com/nar/article-abstract/3/6/1463/2359339)
tion process a series of 9 discrete bands of DNA whose molecular weights correspond to integral multiples of a monomeric subunit 185 base pairs in length. As the enzymatic process proceeds, the higher multiple fragments decrease with a concomitant increase in the relative abundance of the monomeric form. On the left in Fig. 1, we observe the fragments resulting from digestion of interphase nuclei derived from colchicine-treated cultures used in the preparation of metaphase chromosomes. The multimeric fragments generated are identical to those observed upon digestion of mitotic chromosomes, and the pattern of these fragments is indistinguishable from that observed after mild nuclease treatment of a variety of eucaryotic tissues (4-8).

These experiments indicate that the organization of DNA into regularly spaced nucleosomes is not restricted to interphase chromatin. The unit fiber of the mitotic chromosome is likely to result from this nucleosomal structure as well. These data are in accord with previous observations that the repeating subunit can be reconstituted with DNA and histones alone and suggest that the nonhistone proteins are not essential for the assembly or maintenance of nucleosome structure.

Further evidence for the existence of nucleosomes in mitotic chromosomes could be obtained if we could demonstrate the presence of the multimeric nucleoprotein complexes from which the DNA fragments seen in Fig. 1 are derived. This has been readily accomplished in the past using sucrose gradient centrifugation (5, 7, 12). We have performed similar analyses of nucleoproteins by agarose-acrylamide electrophoresis. A linear relationship is obtained between the logarithm of the molecular weight and the migration, providing the ionic strength of the gel buffers is sufficiently low to prevent dissociation of the nucleosomal complex. Metaphase chromosomes were therefore subjected to nuclease digestion, and the resultant nucleoprotein fragments were directly analyzed by gel electrophoresis under conditions in which the proteins remain bound to DNA. At 2% solubilization of DNA, the pattern seen in Fig. 2 is obtained. The predominant peak is that of the monomeric form with lesser amounts of dimer, trimer and tetramer clearly present. To identify these nucleoprotein bands definitively, the nucleoprotein was eluted from the regions of the gel labeled 1-4 and freed of protein by phenol-chloroform extraction. The molecular weights of the resulting DNAs were then determined by a second gel electrophoresis. Peaks 1-4 consisted of relatively pure DNA fragments 185, 370, 560, and 740 base pairs in length. These results are in accord with those obtained by velocity
Figure 2. Polyacrylamide gel electrophoresis of nucleoprotein complexes liberated upon digestion of metaphase chromosomes. Metaphase chromosomes were digested with staphylococcal nuclease to 10% solubilization of DNA. 10 µg of nucleoprotein fragments were applied to a 2.5% acrylamide–0.5% agarose gel. Electrophoresis was carried out as previously described at 200 V for 2 hrs, and the gel was stained with ethidium bromide and photographed. The negative was scanned at 550 nm in a Beckman ACTA Spectrophotometer.

centrifugation of chromatin subunits and provide direct evidence for the liberation of nucleosomes from metaphase chromosomes.

The previous results all tend to confirm the existence of a regular array of nucleosomes on the metaphase fiber, but provide little information as to the internal structure of the nucleosome itself. It has been shown (13, 14) that the individual monomeric subunits obtained from interphase nuclei contain internal cleavage sites accessible to nuclease attack. Digestion of this intranucleosomal DNA results in the liberation of a smaller set of DNA fragments ranging in molecular weights from 45-150 base pairs in length. This highly reproducible set of DNA fragments has been obtained from limit digests of either chromatin, nuclei or isolated nucleosomes from a variety of tissues and reflects the arrangements of histones along the DNA within the nucleosome.
The kinetics of appearance of these fragments following digestion of mitotic chromosomes is shown in Fig. 3. Metaphase chromosomes were digested with nuclease to solubilize 14 and 28% of the DNA. The resistant DNA was freed of protein and analyzed by 6% polyacrylamide gel electrophoresis. At 14% digestion, we observe both the higher molecular weight fragment corresponding to multimers of nucleosomes and the smaller DNAs that result from cleavages within the nucleosomes. As the digestion proceeds, the multimeric DNA disappears with a corresponding increase in the proportion of lower molecular weight fragments. The precision with which the enzymic process results in this highly reproducible set of fragments suggests that they reflect a basic substructure within the nucleosome, which is common to all animal cells examined. The present observations suggest, therefore, that not only is the organization of nucleosomes retained during mitosis, but also that the internal structure of the monomeric subunit is retained.

Attempts to assess the subunit structure of metaphase chromosomes require the preservation of this structure during the purification procedure and digestion. To test the possibility that rearrangement of chromosomal

![Figure 3. Polyacrylamide gel electrophoresis (6%) of DNA liberated upon digestion of metaphase chromosomes and interphase nuclei. Interphase nuclei and metaphase chromosomes were digested with staphylococcal nuclease. The resistant DNA was freed of protein and 10 µg applied to each slot of a 5% polyacrylamide gel. The gel was run as described previously, stained with ethidium bromide, and photographed. Samples from right to left represent 14 and 28% digestion of metaphase chromosomes and 10 and 30% digestion of nuclei.](image)
proteins occurs during purification of isolated chromosomes, preparations of interphase cells and mitotic cells were washed in phosphate buffered saline and then immediately lysed and digested in 1mM Tris-HCl, pH 7.9, 0.1 mM CaCl₂ containing 0.2% Triton. The multimeric fragments generated from both cell types were identical to those obtained from purified nuclei or chromosomes (data not shown).

DISCUSSION

Analysis of the products of nuclease digestion allows us to discern two levels of organization of DNA within the metaphase chromosome. Early in the digestion process, a series of nucleoprotein fragments is liberated that reflect the regular array of nucleosomal subunits, characteristic of the interphase nucleus as well. Further digestion results in the liberation of a series of smaller DNA fragments and reflects an aspect of structure within the monomeric subunit itself. It would appear therefore that although significant morphologic changes occur within the chromatin complex during mitosis, the basic subunit structure of the chromatin fiber is retained by the metaphase chromosome.

Previous attempts to elucidate the structure of the mitotic chromosome have relied heavily on electron microscopic observations. From the present study, we confirm that the 7-9-nm beads observed in the electron microscope (11) result from the organization of DNA into nucleosomal subunits. The native 23-30-nm fiber therefore probably results from higher order folding of the 10-nm unit fiber and reflects an aspect of subunit-subunit interaction that may be unique to the mitotic chromosome.

ACKNOWLEDGMENTS

This research was supported by Grants CA-02332 and 16346 awarded by the National Cancer Institute, DHEW.

REFERENCES
