REPEATED STRUCTURE OF CHROMATIN IN METAPHASE NUCLEI OF *PHYSARUM*

Volker M. VOGT and Richard BRAUN
Institute of General Microbiology, University of Bern, Altenbergrain 21, 3013 Bern, Switzerland

Received 9 February 1976

1. Introduction

Several lines of evidence suggest that eucaryotic chromatin is organized in a repetitive structure. Digestion of nuclei with endogenous nuclease [1], or with appropriate concentrations of micrococcal nuclease [2] leaves most of the DNA in fragments of about 200 nucleotide pairs, or multiples thereof. Two molecules each of histones F2a, F2b, F3, and F4 appear to condense with each 200 nucleotide pair length of DNA to form one 'nucleosome' of chromatin [3,4]. These nucleosomes can be visualized as contiguous beads of approximate diameter 60–130 Å in the electron microscope [3,5–7]. The roles of histone F1 and of other proteins in the organization of chromatin structure are not yet clear. Among the macromolecular changes associated with mitosis are cessation of transcription [8,9] and modifications of histones [10–13]. In particular, phosphorylation of histone F1 has been implicated in chromatin condensation [14]. Although the total histone content of mitotic and interphase chromatin is the same, it is not known in these cases whether or not the histones are arranged in the same supramolecular structure. In the experiments presented, we addressed ourselves to the question, are the nucleosomes characteristic of interphase also present at mitosis.

2. Materials, methods and results

In the surface cultures, *Physarum polycephalum*, an acellular slime mold, grows as a syncytium containing up to 10^8–10^9 nuclei. The nuclei divide by mitosis with an exact, natural synchrony. Since the nuclear membrane never disappears, it is possible to isolate large numbers of synchronous nuclei at all stages of the cell cycle. We show here by analyzing DNA after digestion of such nuclei with micrococcal nuclease that the basic repetitive structures of metaphase and of interphase chromatin are indistinguishable. Fig.1 shows the patterns of DNA obtained. After digestion with nuclease at the highest concentration (1c and 1f) most of the DNA migrates as a single band. Five times less nuclease leaves a series of bands containing DNA with mol. wts. that are multiples of this 'monomer' band. At the lowest nuclease concentration little digestion takes place. No differences in metaphase and interphase DNA patterns are apparent. Identical patterns are also obtained if nuclei are isolated 5 min before or 5 min after mitosis, or during the middle of the S phase (not shown). As seen in fig.1g and 1h, the size of the DNA in *Physarum* nucleosomes is closely similar to the size of protected fragments obtained from mouse cell nuclei, which we assume to be multiples of 200 nucleotide pairs as reported by Noll [2] for rat cells.

To investigate if the percentage of DNA protected against nuclease differs in interphase and metaphase, nuclei were digested from plasmodia that had been labeled with [^{3}H]thymidine. After incubations identical to those described for fig.1b, 1c, 1e and 1f, the nuclease was inhibited with 0.02 M EDTA and the nuclei lysed by dilution into 2% sodium dodecyl sulfate. Aliquots of the lysates were then analyzed directly by gel filtration on Sephadex G-200. In all experiments two well separated peaks were obtained: one close to the excluded volume, the other close to the total bed volume. Table 1 lists the percentages of radioactivity in the excluded volume, i.e. in fragments larger than about 100 nucleotide pairs, for each gel filtration. For both nuclease concentrations, the

North-Holland Publishing Company – Amsterdam

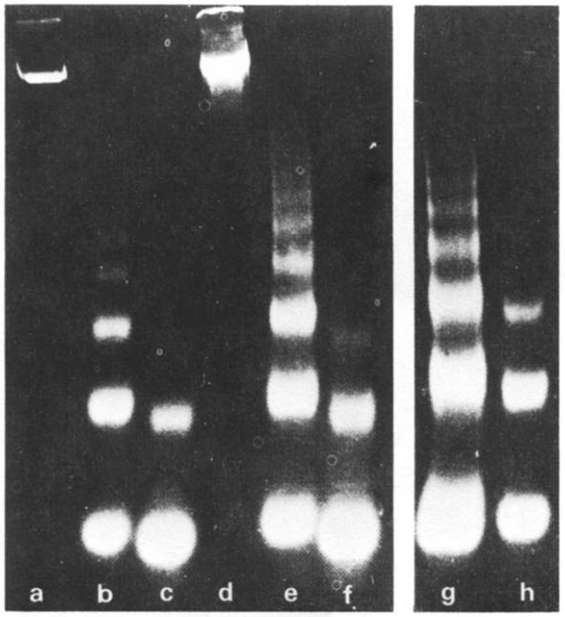

Fig.1. Polyacrylamide slab gel electrophoresis of DNA fragments. Physarum surface cultures were grown as described by Daniel and Baldwin [15]. Near the expected time of mitosis, a small part of one plasmodium was removed every 3 min and observed in a phase contrast microscope. At mitosis (± 2 min) the plasmodium was scraped into 40 ml ice cold lysis buffer (0.01 M Tris pH 7.0, 0.01 M $CaCl_2$, 0.25 M sucrose, 0.1% Triton X-100) and the nuclei purified rapidly by standard procedures [16] with omission of the centrifugation through 0.88 M sucrose. The nuclei from P815 mouse mastocytoma cells or from *Physarum* were each dispersed in 1.5 ml 0.02 M Tris, pH 7.8, 0.06 M KCl, 0.015 M NaCl, 0.001 M $CaCl_2$. One half ml aliquots of each sample, or about 2×10^7 nuclei, were digested for 5 min at 37°C with 0.02 μg, 0.1 μg, or 0.5 μg micrococcal nuclease (Sigma Biochemicals). The DNA was extracted with phenol, concentrated by ethanol precipitation, and then electrophoresed in a 3 mm thick slab gel of 2.5% polyacrylamide [2]. The bromphenol blue tracking dye ran slightly ahead of the most rapidly migrating DNA fragment. The DNA bands were visualized after staining with ethidium bromide under an ultraviolet lamp. (a–c), *Physarum* interphase nuclei; (d–f) *Physarum* metaphase nuclei; (g) mouse nuclei; (h) *Physarum* nuclei. (a) and (d) 0.02 μg nuclease; (b) and (e), 0.1 μg nuclease; (c, f, g,) and (h) 0.5 μg nuclease. Columns (g) and (h) are from a different slab gel than (a–f).

chromatin in interphase and in metaphase nuclei appears to be equally accessible to micrococcal nuclease.

Thus we conclude that the basic repetitive structure of chromatin does not change as *Physarum* nuclei pass through mitosis. Similar conclusions have also been reached by others for the chromatin of higher eukaryotes (L. Compton, R. Hancock, P. Oudet, and P. Chambon, personal communication; G. B. Howze, A. W. Hsie, and A. L. Olins, personal communication).

Table 1
Quantitation of digestion

Nuclease (μg)	% DNA in excluded volume	
	Interphase nuclei	Metaphase nuclei
0.1	95	88
0.5	83	80

Nuclei from plasmodia labeled with [^{3}H]thymidine were digested as described in fig.1, and then dodecyl sulfate lysates of the nuclei were analyzed by gel filtration on a column of Sephadex G-200.

Acknowledgements

We thank P. Nobis and J. L. Compton for helpful discussions, and H. Gautschi for the mouse cell nuclei. This research has been supported by grant 3.750.72 of the Swiss Science Foundation.

References

[1] Hewish, D. R. and Burgoyne, L. A. (1973) Biochem. Biophys. Res. Com. 52, 504–510.
[2] Noll, M. (1974) Nature 251, 249–251.
[3] Oudet, P., Gross-Bellard, M. and Chambon, P. (1975) Cell 4, 281–300.
[4] Kornberg, R. D. and Thomas, J. O. (1974) Science 184, 865–868.
[5] Olins, A. L. and Olins, D. E. (1974) Science 183, 330–332.
[6] van Holde, K. E., Sahasrabuddhe, C. G., Shaw, B. R., van Bruggen, E. F. J. and Arnberg, A. C. (1974) Biochem. Biophys. Res. Com. 60, 1365–1370.
[7] Griffith, J. (1975) Science 187, 1202.
[8] Prescott, D. M. and Bender, M. A. (1962) Exp. Cell Res. 26, 260–268.
[9] Mittermayer, C., Braun, R. and Rusch, H. P. (1964) Biochim. Biophys. Acta 91, 399–405.
[10] Lake, R. S., Goidl, J. A. and Salzman, N. P. (1972) Exp. Cell Res. 73, 113–121.