CHROMATIN STRUCTURE OF *PHYSARUM POLYCEPHALUM* PLASMODIA AND AMOEBAE

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1. Introduction

Nucleosome repeats of chromatin in higher eukaryotes contain DNA fragments which are multimers of a 190–210 base pair subunit [1], while the DNA repeat length in lower eukaryotes is markedly smaller [2,3]. Both higher and lower eukaryotes, however, contain a similar core particle with about 140 base pairs of DNA [2,3].

While there are significant differences in DNA repeat lengths between organisms, variable repeats are also observed among different cells of a given organism. Shorter DNA repeats are found in actively dividing cells compared to chromatin of mature tissues [1,4]. Similarly, transcriptionally active chromatin shows shorter DNA repeats than chromatin of transcriptionally dormant cells [5,6]. In contrast, DNA repeat length does not vary among interphase and mitotic nuclei [7,8].

The acellular slime mold *Physarum polycephalum* is widely used to study biochemical changes during differentiation. In the present study, we compared DNA repeat lengths of the subunits released after micrococcal nuclease digestion of chromatin from the slowly dividing haploid nuclei in *Physarum* amoebae (mitotic cycle 72–96 h) and from the rapidly dividing nuclei in diploid plasmodia (mitotic cycle 8–9 h). In addition, we incubated nuclei from plasmodia and amoebae with DNase I and analyzed the released DNA fragments on gels.

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2. Materials and methods

Physarum polycephalum plasmodia (strain M_3C VIII) and amoebae (strain CL_d) were cultured and nuclei were isolated as in [9–12].

Micrococcal nuclease digestions were carried out as in [8] for 30 s to 5 min at 37°C with 2 μg/ml of enzyme at a DNA concentration of about 500 μg/ml. For digestions with pancreatic DNase (DNase I), nuclei were suspended in 10 mM Tris–HCl, pH 8.0, 10 mM NaCl, 3 mM $MgCl_2$ at a DNA concentration of 300–400 μg/ml and incubated with 20 μg/ml of DNase I at 37°C for 30 s.

Enzyme activity was stopped by adding 0.1 vol. 0.2 M Na_3EDTA followed by incubation with proteinase K in the presence of 1% SDS. DNA was extracted with phenol and concentrated by ethanol precipitation at –20°C overnight. Pelleted DNA was redissolved in a small volume of 0.1 × SSC and incubated with RNase and proteinase K, followed by deproteinization with phenol and concentration by ethanol precipitation.

Electrophoresis of native DNA in polyacrylamide–agarose slab gels was carried out as in [1]. About 2 μg DNA in 10 mM Tris–HCl, pH 7.8, 1 mM Na_3EDTA containing 6% sucrose was layered into each sample well of the gels.

Electrophoresis of DNA fragments under denaturing conditions was carried out on 10% polyacrylamide–7 M urea slab gels [14] after denaturing the DNA in 50% formamide for 10 min in a boiling water bath.

Gels were stained with 0.5 μg/ml ethidium bromide in running buffer for 30 min and photographed under

short wavelength ultraviolet light through a red filter. Negatives from photographs were scanned in a Beckman spectrophotometer.

3. Results and discussion

Isolated nuclei from *Physarum polycephalum* plasmodia and amoebae were incubated with micrococcal nuclease until either 3%, 8% or 15% of the DNA became acid soluble. Figure 1 shows a polyacrylamide–agarose slab gel with nuclease digests of plasmodial and amoebal chromatin. The lengths of the DNA fragments representing dimers to hexamers were calculated by least-squares analysis of the logarithm of the base pairs versus their migration

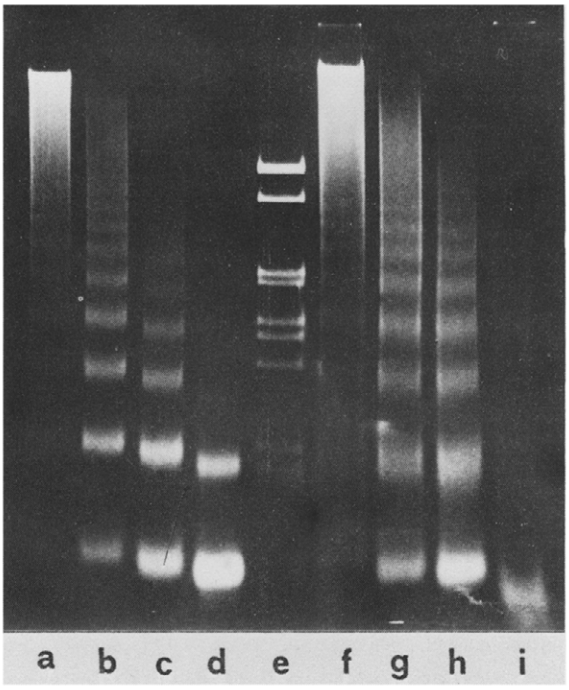

Fig.1. Chromatin from *Physarum polycephalum* plasmodia and amoebae degraded by micrococcal nuclease. The DNA fragments released were separated on a 2% acrylamide–0.5% agarose slab gel. For calibration, Hae III fragments of PM 2 were coelectrophoresed. Panels a, b, c, d: digests of plasmodial chromatin to either 3%, 8%, 15% or 50% acid solubility; panel e: PM 2 digested with Hae III; panels f, g, h, i: digests of amoebal chromatin to either 3%, 8%, 15% or 50% acid solubility.

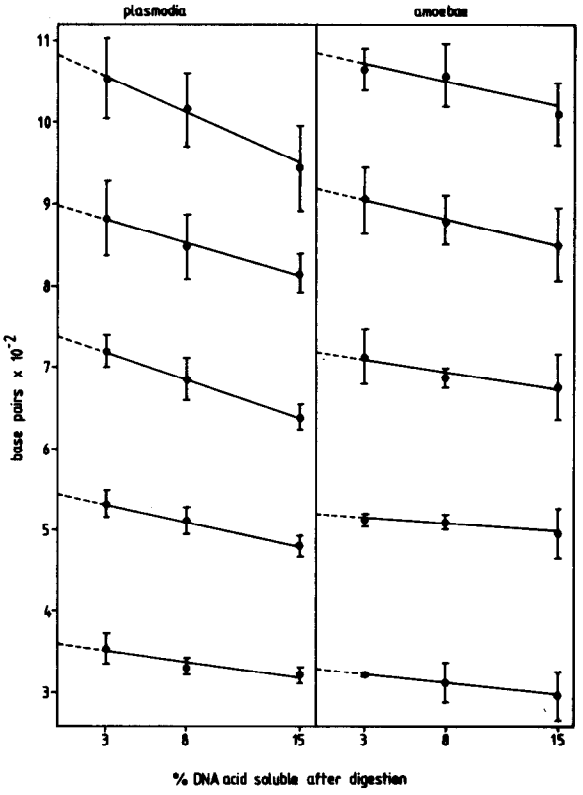

Fig.2. Chromatin from *Physarum* plasmodia and amoebae were digested with micrococcal nuclease to either 3%, 8% or 15% acid solubility. The DNA fragments released were separated on gels together with PM 2 DNA fragments (Hae III digest) as shown in fig.1. From the relative mobilities of the bands, the sizes of the *Physarum* DNA fragments (dimers to hexamers) were calculated and plotted as a function of the extent of nuclease digestion.

distance, using Hae III fragmented DNA of PM 2 as a standard.

With increasing digestion, the sizes of the *Physarum* DNA fragments became smaller. In fig.2, the decrease of the DNA fragment sizes from plasmodia and amoebae are plotted as a function of the amount of DNA which became acid soluble during nuclease digestion. The large decrease in fragment lengths of higher oligomers with increasing digestion time was rather unexpected. A possible explanation would be a movement of nucleosomes along the DNA during the degradation process [15,16].

To determine the true DNA repeat length of

Table 1
DNA fragment length of oligomers

Plasmodia (4)[a]			Amoebae (3)[a]		
Band no.	Base pairs	Base pairs/ band no.	Band no.	Base pairs	Base pairs/ band no.
6	1085	180.8	6	1086	181.0
5	896	179.2	5	917	183.4
4	739	184.8	4	718	179.5
3	545	181.7	3	518	172.7
2	360	180.0	2	330	165.0
Mean DNA repeat length: 181.3 ± 2.2			Mean DNA repeat length: 176.3 ± 7.5		

[a] number of independent gels that were measured

The lengths of DNA fragments obtained following micrococcal nuclease digestion of nuclei from *Physarum polycephalum* plasmodia and amoebae. Lengths are obtained using DNA fragments of PM 2 digested with Hae III for calibration. DNA lengths of multimers were determined from relative mobilities of the centers of the bands after digesting chromatin to different degrees of acid solubility and extrapolation to 0% acid solubility as shown in fig.2. The DNA repeat length was obtained by dividing the fragment lengths by the corresponding band numbers

Physarum chromatin, the DNA fragment lengths after nuclease digestions to various extents were extrapolated to 0% acid solubility of the DNA as indicated in fig.2. The DNA fragment lengths of dimers to hexamers from plasmodial and amoebal chromatin digests are summarized in table 1. Dividing the fragment lengths by the corresponding band numbers gives a DNA repeat length of 181 ± 2 base pairs for plasmodial chromatin and 176 ± 8 base pairs for amoebal chromatin. The DNA repeat length of amoebal chromatin is only marginally smaller than that of plasmodial chromatin. However, it is worth stating that tetramers to hexamers of amoebal chromatin have DNA fragment lengths very similar to plasmodial chromatin. The difference of the two arises from dimers and trimers, where the fragments from amoebal chromatin are markedly shorter than those from plasmodial chromatin. Therefore, it is likely that plasmodia and amoebae have the same basic DNA repeat length of about 181 base pairs and that small oligomers (dimers and trimers) are degraded faster to shorter fragments by micrococcal nuclease in amoebae. Consistent with this assumption is the fact that amoebal chromatin, after nuclease digestion to 50% acid solubility, shows a series of DNA fragments shorter than 140 base pairs (fig.3). In contrast, the plasmodial chromatin is degraded upon prolonged nuclease digestion to a fragment of about 159 base pairs, a value reported in [17]. A core fragment of 140 base pairs as in other eukaryotes [18–20] is not obtained with chromatin from *Physarum* plasmodia. Chromatin from amoebal nuclei is directly degraded to fragments shorter than 140 base pairs. This result suggests that amoebal chromatin lacks a more stable digestion product in contrast to plasmodial chromatin, where 159 base pairs of DNA seem to form a core particle with the 8 histones present in a nucleosome.

The DNA repeat length of 181 base pairs obtained for plasmodial chromatin is slightly different from values reported by others [1,17]. A different gel calibration and the extrapolation to 0% acid solubility of the DNA to determine the basic repeat length may account for the differences. However, all reports suggest a smaller DNA repeat length of chromatin from *Physarum polycephalum* compared to higher eukaryotes. In addition, the DNA repeat length in *Physarum* is independent of the duration of the mitotic cycle and of the ploidy of the nuclei.

To obtain some information about the internal structure of nucleosomes of chromatin from *Physarum* plasmodia and amoebae, nuclei from both differentiation stages were incubated with pancreatic DNase

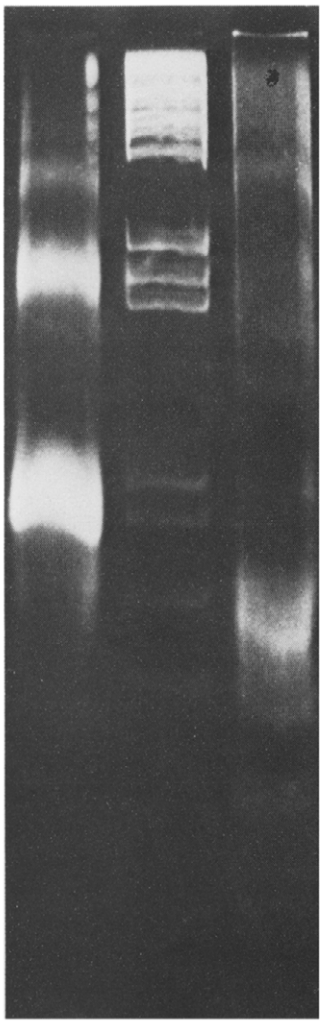

Fig.3. Nuclei from *Physarum* plasmodia and amoebae were incubated with micrococcal nuclease until 50% of the DNA became acid soluble. After extraction, the DNA fragments were separated together with DNA fragments of a Hae III digest of PM 2 on a 5.5% acrylamide–0.5% agarose gel. The lengths of the 4 lower bands of PM 2 are 95, 120, 152 and 167 base pairs as determined in [3]. a, plasmodial DNA fragments; b, Hae III digest of PM 2; c, amoebal DNA fragments.

Fig.4. Nuclei from *Physarum* plasmodia and amoebae were incubated with pancreatic DNase (DNase I) until 15–20% of the DNA became acid soluble. After denaturing the extracted DNA fragments in 50% formamide at 98°C for 10 min, they were separated on a 10% acrylamide–7 M urea gel. a, DNA fragments from rat liver nuclei; b, fragments from plasmodial nuclei; c, fragments from aemoebal nuclei.

(DNase I). Incubation of rat liver chromatin with DNase I and analysis of the DNA released under denaturing conditions revealed a series of fragments which were multiples of 10 nucleotides [21]. As fig.4 shows, the released DNA fragments from both plasmodial and amoebal chromatin show a regular pattern of single stranded DNA fragments identical to that of rat liver. Although amoebal chromatin shows no stable nuclease resistant core, a similar association of DNA and histones as in other eukaryotic chromatin is observed [21,22].

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