SUBUNIT STRUCTURE OF *PHYSARUM POLYCEPHALUM* CHROMATIN

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1. Introduction

Digestion of animal chromatin [1–4] as well as chromatin from higher plants [5] and yeast [6] by exogenous bacterial nuclease has led to the conclusion that chromatin has a regular subunit structure. It is however still important to establish whether this structure is common especially among organisms in which the histone pattern is not identical with that of higher plants and animals. We report here studies on the chromatin organization in a true slime mold *Physarum polycephalum* which belongs to a very primitive group of eukaryotes and is widely used as a model organism especially in mitotic studies [7].

2. Materials and methods

2.1. *Cultures*

The strain M_3C IV of *Physarum polycephalum* was maintained in submerged, shaken culture according to Daniel and Baldwin [8]. For some experiments DNA was labelled by adding 5 μCi/ml of [^{3}H]thymidine to the growth medium for a 24 h period.

2.2. *Preparation of nuclei*

Nuclei of *Ph.polycephalum* were prepared from cultures in log phase of growth according to the method described by Jockusch and Walker [9]. Calf thymus nuclei were prepared as follows: 20 g of calf thymus were homogenized with 180 ml of buffered sucrose (0.25 M sucrose, 0.01 M Tris-HCl pH 7.8, 3 mM $MgCl_2$). The homogenate was filtred through double cheese cloth and the crude nuclear pellet was sedimented by centrifugation for 10 min at 1000 *g*. The pellet was washed twice with 4 vol. of buffered sucrose, suspended in 30 ml of this medium then layered over the solution of 2 M sucrose, 0.01 M Tris-HCl pH 7.8, 3 mM $MgCl_2$ and centrifuged for 1 h at 22 000 rev/min in the SW 27 Spinco rotor. The final pellet was resuspended in 15 ml of buffered sucrose medium and sedimented by centrifugation for 10 min at 1000 *g*.

2.3. *Digestion of nuclei*

Both *Ph. polycephalum* and calf thymus nuclei were incubated in a solution of 0.25 M sucrose, 0.001 M Tris HCl pH 7.8 and 1 mM Ca^{2+} at 37°C to which micrococcal nuclease (Worthington) was added to a concentration of 200 units/ml. The DNA concentration was 0.2–0.3 mg/ml in the case of *Ph. polycephalum* and 0.5–1.0 mg/ml in the case of calf thymus nuclei. The reaction was stopped by adding NaEDTA to a final concentration of 10 mM and chilling on ice. The suspension was then centrifuged for 10 min at 1000 *g* and the supernatant was used for further experiments. In the case of calf thymus DNA the extraction procedure was applied to the whole suspension after digestion of nuclei.

2.4. *Preparation and polyacrylamide gel electrophoresis of DNA fragments*

DNA was extracted by the method of Stern [10] using digestion with pronase and pancreatic ribonuclease. Electrophoresis of DNA in 3% acrylamide gels was carried out according to Loening [11]. Ethidium bromide was added directly to the sample applied to the gel. After electrophoresis for 30 min gels were photographed under u.v. light.

2.5. *Sucrose gradient centrifugation*

To the supernatants obtained after centrifugation

of digested nuclei at 1000 *g* were added 1.5 vol. of 0.2 mM NaEDTA (pH 7.2) and 0.75 ml of this solution was layered over 17.45 ml of a 5–20% (w/w) isokinetic sucrose gradient containing 0.2 mM NaEDTA, pH 7.2 (c_t = 5%, c_r = 27.3%, V_m = 14.2 ml). Gradients were centrifuged at 5°C in the SW 27 (5/8 × 4) Spinco rotor at 25 000 rev/min for 16 h and analysed by collecting 15 or 10 drop fractions and measuring the absorbance at 260 nm (calf thymus) or ^{3}H radioactivity (*Ph. polycephalum*).

2.6. *Estimation of DNA, RNA and protein*

DNA was determined by the method of Burton [12]. RNA after alkaline hydrolysis was determined spectrophotometrically at 260 nm and protein was estimated by the method of Lowry et al. [13].

3. Results and discussion

Limited digestion of isolated nuclei of *Ph. polycephalum* with micrococcal nuclease followed by short centrifugation releases a considerable part of the nuclear DNA into the 1000 *g* supernatant. After 2.5 min of digestion roughly 2/3 of the DNA is released from nuclei in the form of soluble nucleoprotein.

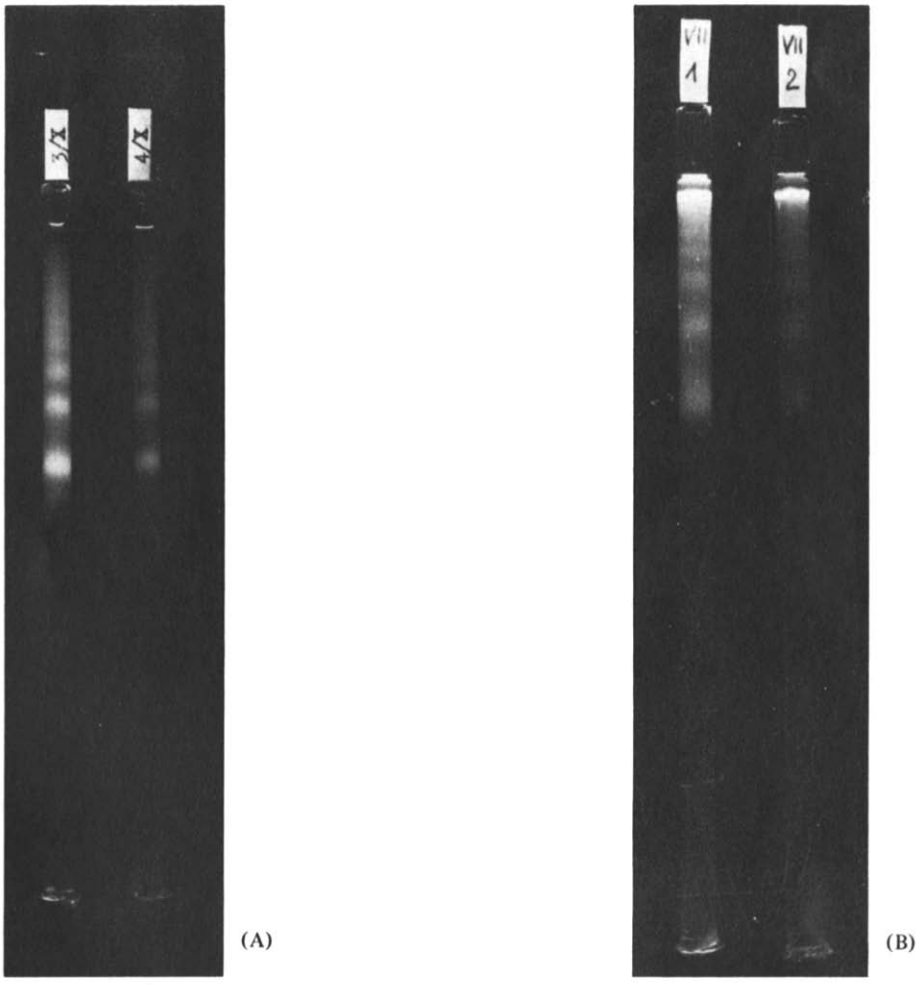

Fig.1. 3% polyacrylamide gel electrophoresis of nuclear DNA digestion products prepared from *Ph. polycephalum* post-nuclear, 1000 *g* supernatant (A) and calf thymus nuclei (B). Digestion (A and B) with 200 units/ml of micrococcal nuclease for 2.5 min at 37°C. In both A and B, gels with larger and smaller loads are shown.

Table 1
Digestion of *Ph. polycephalum* nuclei with micrococcal nuclease

Time of incubation with nuclease	Insoluble DNA in the pellet (μg)	DNA in 1000 *g* supernatant (μg)
0 (Control)	135	11
40 sec	95	50
1.0 min	79	74
2.5 min	57	95

Ph. polycephalum nuclei were suspended in 5.0 ml of incubation mixture and digested with 200 units/ml of micrococcal nuclease at 37°C. For analysis one ml samples were taken at each time and ice-cold NaEDTA was added immediately to 10 mM final concentration. Samples were then centrifuged at 1000 *g* for 10 min and DNA was determined in pelleted nuclei and supernatants.

The nature of the DNA isolated from 1000 *g* supernatant after 2.5 min of digestion as revealed by polyacrylamide gel electrophoresis is shown in fig.1. Presented for comparison is the gel pattern of DNA released after 2.5 min of digestion of calf thymus nuclei with the same concentration of micrococcal nuclease. In this case the DNA was extracted from the whole incubation mixture without prior centrifugation of nuclei which accounts for the relatively large band seen at the top of the gel.

It can be seen that almost all of the *Ph. polycephalum* DNA enters the gel showing a very regular pattern of migration. This pattern strongly resembles the pattern of DNA bands obtained after digestion of calf thymus nuclei with micrococcal nuclease. In both cases the plots of the logarithms of band number versus distance migrated on the gel form straight lines (fig.2).

Such a regular pattern can only be produced when the larger bands are integral multiples of the basic monomer band. In the case of *Ph. polycephalum* the lowest distinct band, which we assume to be the basic monomer, migrates slightly faster than the corresponding lowest band of calf thymus. It may indicate that the size of *Ph. polycephalum* monomeric DNA although comparable to the size of calf thymus monomeric DNA is slightly smaller.

From gel electrophoresis it is thus clear that DNA in *Ph. polycephalum* chromatin is arranged in regular and repeating size classes, resembling strongly the pattern of DNA organization found in chromatins of higher eukaryotes.

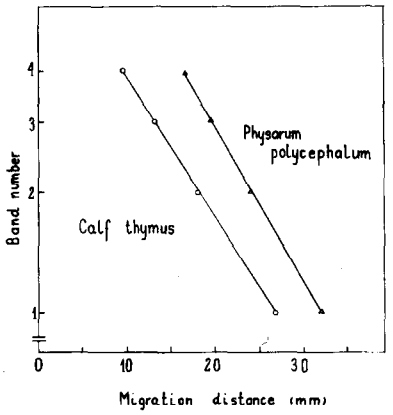

Fig.2. Log plots of band number versus mobility on the gel. (△ – △) *Ph. polycephalum* digest. (○ – ○) Calf thymus digest. Results are from fig.1.

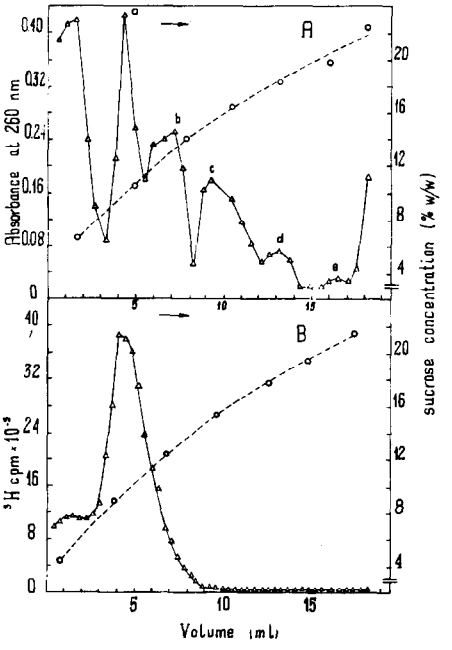

Fig.3. 5–20% (w/w) isokinetic sucrose gradient sedimentation of 1000 *g* post-nuclear supernatants after digestion of *Ph. polycephalum* (B) and calf thymus (A) nuclei with 200 units/ml of micrococcal nuclease for 2.5 min. (△ – △) Absorbance at 260 nm (calf thymus). (△ – △) ^{3}H radioactivity (*Ph. polycephalum*). (○—○) Sucrose concentration. Arrows give the direction of sedimentation.

The nucleoprotein released after limited digestion of *Ph. polycephalum* nuclei has a low protein: DNA ratio of 1.2–1.3 and contains all the histone bands characteristic of isolated chromatin (results not shown). The DNA : RNA ratio in this nucleoprotein is about 2.

The profiles of 5–20% isokinetic sucrose gradient centrifugation of *Ph. polycephalum* and calf thymus 1000 *g* supernatants obtained after 2.5 min of digestion of nuclei are shown in fig.3. In the case of *Ph. polycephalum* the radioactivity of DNA labelled with [^{3}H]thymidine was monitored instead of absorbance at 260 nm to avoid the influence of absorbance from RNA. A 2.5 min digest of calf thymus nuclei gives a characteristic pattern of digestion products. Five peaks (a–e) corresponding to the basic subunit and its multimers [14] can be distinguished in this profile. Identical sucrose gradient centrifugation of a *Ph. polycephalum* 2.5 min digest gives a distinct and relatively sharp peak with a slightly broader right arm and a maximum corresponding to the calf thymus basic subunit (peak a). There are however no peaks in the region of the calf thymus higher multimers (peaks c–e). Since gel electrophoresis of *Ph. polycephalum* DNA extracted from a 2.5 min digest reveals the existence of higher multimers (probably up to heptamers) we conclude that the higher nucleoprotein multimers may be agreggated with some RNA or additional non-histone protein and sediment in sucrose gradient faster than corresponding multimers of calf thymus chromatin.

The results presented here indicate that DNA in *Ph. polycephalum* chromatin is arranged in repeating fragments of a size comparable to that of similar DNA fragments from higher eukaryotes. From data of sucrose gradient sedimentation it is very probable that the complete nucleoprotein subunit of *Ph. polycephalum* chromatin has an S value very close to the sedimentation constant of the subunit of calf thymus chromatin.

Myxomycetes of which *Ph. polycephalum* is a representative belong to a very simple and evolutionarily old group of eukaryotes, perhaps even more primitive than fungi. Together with the data given by Lohr and Van Holde [6] reporting the regular pattern of DNA fragments after digestion of yeast chromatin this work shows that the idea of subunit organization of chromatin can be extended to the most primitive forms of eukaryotes.

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