BIOCHEMICAL EVIDENCE FOR A DNA REPEAT LENGTH IN THE CHROMATIN OF *DICTYOSTELIUM DISCOIDEUM*

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1. Introduction

Digestion of eukaryote chromatin with nucleases produces a series of discrete DNA fragments [1–3]. These results support the model of eukaryotic chromatin as a regularly repeating structure resembling beads on a string [4,5]. The DNA in the repeating subunits, nucleosomes, is apparently tightly folded around a protein core of eight histone molecules [6].

The cellular slime mold, *Dictyostelium discoideum*, is widely used to study biochemical changes occurring during differentiation [7]. Modulation of gene activity accompanies the morphological changes occurring during the life cycle of *D. discoideum* [8]. We are presently examining the structure of chromatin and the changes in nuclear proteins during differentiation. We have isolated basic nucleoproteins from *D. discoideum* which differ considerably from calf thymus histones [9,10]. Consequently, we were interested to discover whether the chromatin structure of *D. discoideum*, as deduced from experiments with micrococcal nuclease, showed similarities to chromatin of higher eukaryotes.

2. Materials and methods

D. discoideum cells (strain Ax-3) were cultured and nuclei isolated as described [10].

Incubation buffer (0.6 ml; 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$ in 20 mM Tris–HCl, pH 7.8) was added to 0.4 ml nuclei. Incubation was with 150 units micrococcal nuclease (Worthington) for 5 min at 37°C. The reaction was stopped with 0.2 ml 200 mM EDTA, then 0.2 ml 10% (v/v) sodium *N*-lauroyl sarkosine (Sarkosyl) added. An equal volume (1.4 ml) of freshly distilled phenol saturated with sample buffer was added and the mixture vortexed and centrifuged. The upper phase was mixed with the same volume of phenol and centrifuged. Two volumes of ethanol were added to the upper phase and the DNA precipitated out overnight at –20°C. The sediment following centrifugation was resuspended in 70% ethanol–30% gel sample buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.8) and recentrifuged. The sediment was resuspended in 100 μl gel sample buffer containing 1 μg/ml RNAase. Finally, 20 μl 80% glycerine, 4% SDS and 0.1% bromphenol blue in gel sample buffer was added and the DNA fragments separated on acrylamide–agarose gels according to Compton et al. [11]. Gel slabs were stained with ethidium bromide and photographed under ultraviolet light.

3. Results and discussion

Figure 1 shows gels of DNA fragments obtained following micrococcal nuclease digestion of *D. discoideum* and *Physarum* [12] nuclei and rat liver chromatin [11,13]. The *D. discoideum* pattern differs from the other two in a number of aspects.

Two bands are always present in the monosome region of the *D. discoideum* gels. The larger monomer may be a precursor of the smaller monomer since longer digestion times do tend to reduce the con-

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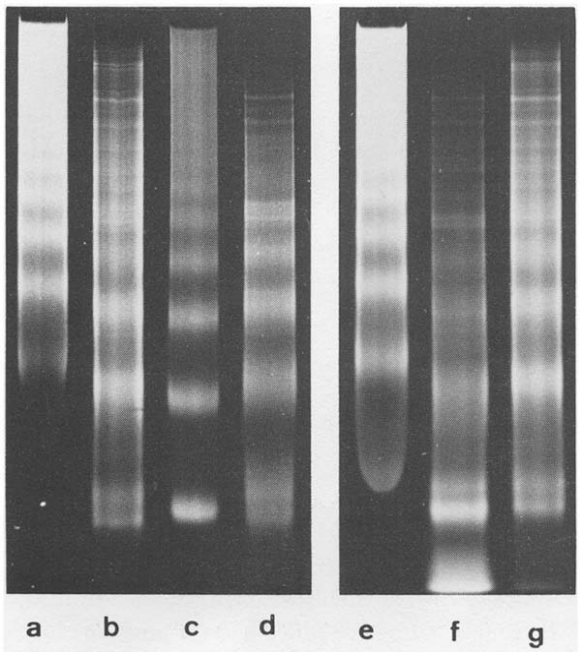

Fig.1. Polyacrylamide–agarose (2.0% and 0.5%) gel electrophoresis of micrococcal nuclease digests of rat liver chromatin (a,e), *D. discoideum* nuclei (b,d,f,g) and *Physarum* nuclei (c). Length of rat liver DNA fragments according to [13] (e.g., monomers 183 ± 15).

centration of the former relative to the latter. However, they may exist in chromatin as independent structures because the two bands are found even after very short digestion times (cf. [14]).

Some of the multimers, instead of presenting a single diffuse band, seem to consist of more than one band (figs 1, 2). The trimer, for example, contains up to four bands (fig.2) and the pentamer clearly contains two bands. The relationship of these bands to the monomers is at present unclear, although the presence of a single diffuse dimer band suggests that if two different sizes of nucleosome (e.g., different spacer sizes) exist they are not in separate tandem groups (i.e., supports a generally dispersed organization).

A number of narrow bands are found at the top of the gels, the significance of which is unknown. They are not observed in controls or following minimal digestion and therefore appear to be products of nuclease treatment.

The sizes of *D. discoideum* DNA fragments were determined using *Hae*III restriction fragments of

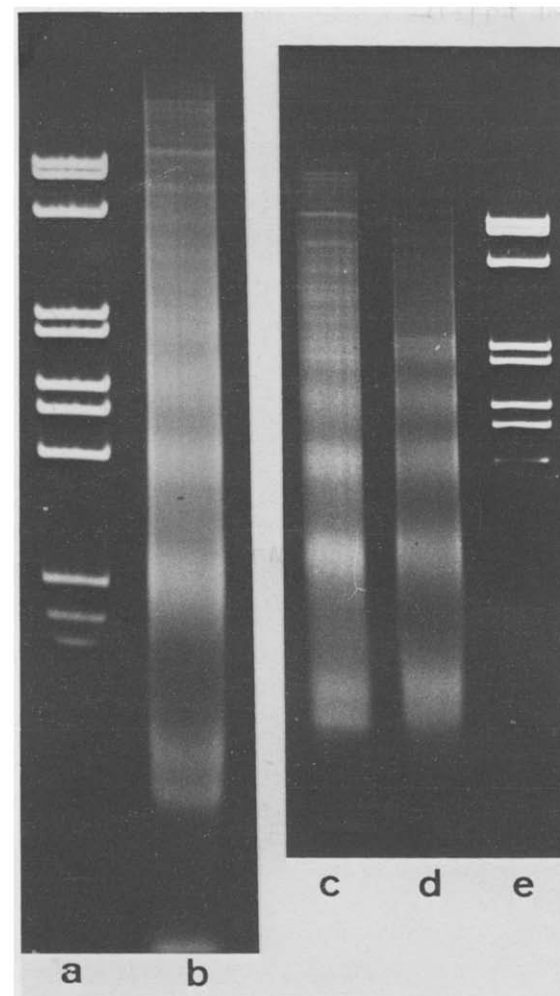

Fig.2. As in fig.1. *Hae*III digests of PM2 DNA (a,e), micrococcal nuclease digests of *D. discoideum* nuclei (b,c,d).

PM2 DNA and micrococcal nuclease digests of rat liver DNA to calibrate the gels (fig.2). The results indicate that the nuclease products have an average repeat length of ca. 170 base pairs, as compared with *Aspergillis* (154 base pairs [15]), *Neurospora* (170 base pairs [17]) and *Physarum* (170 base pairs [11]). However, the picture is complicated by the presence of multiple bands (see above). Hence, the larger monomer is apparantly ca. 175–180 base pairs and so can hardly be derived from a 170 base pair repeat. The larger of the two pentamer bands does have a repeat length approaching 180 base pairs.

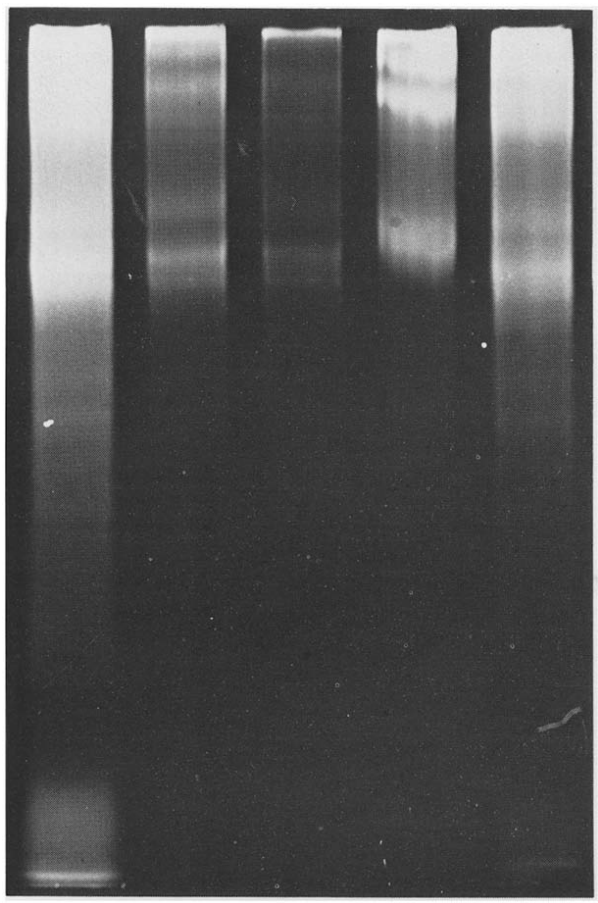

Fig.3. Polyacrylamide–agarose (5.5% and 0.5%) gel electrophoresis of micrococcal nuclease digests of *D. discoideum* and *Physarum* (second from right) nuclei.

The second monomer is ca. 155–160 base pairs and may represent the so-called nucleosome core. A core size of 160 base pairs has recently been reported for the great majority of polynucleosomes in bovine thymus and rat liver [13,16], in contrast to the previous estimation of 140 base pairs (e.g., [11]). The bovine thymus cores are processed further to 140 pairs and small DNA fragments. Similarly 5.5% acrylamide gels (fig.3) separate a third *D. discoideum* band (between 140 and 150 base pairs, depending on the size assumed for the smaller monosome) and a number of smaller DNA fragments.

Hence, *D. discoideum* chromatin has a structure resembling other eukaryotes. Like *Neurospora, Aspergillis* and *Physarum* the DNA repeat is shorter than in higher eukaryotes. Noll [17] and Morris [15] have related this to the lower content of lysine residues in H1 histones of *Neurospora* and *Aspergillis* which may cause them to bind to a correspondingly shorter DNA length. The 'fraction 1' lysine-rich basic nucleoprotein from *D. discoideum* contains 19.6 mol lysine as against 26.8 mol lysine/100 mol total residues in calf thymus H1 [10].

Table 1
The lengths of DNA fragments obtained following micrococcal nuclease digestion of *D. discoideum* nuclei

	Length of multiples (base)			
Multiple	Calibration			
	*Hae*III	PM2 digest	Rat liver	Variation
5 A	890 (179)	890 (178)	875 (175)	
B	850 (170)	845 (169)	830 (166)	
4	675 (169)	685 (171)	670 (168)	± 45
3	510 (170)	510 (170)	505 (168)	± 45
2	347 (170)	338 (169)	327 (164)	± 25
1 A	185	177	183	± 8
B	162	158	154	± 8

Lengths were determined using DNA fragments of PM 2 (*Hae*III digest) and rat liver (micrococcal nuclease digest) to calibrate the gels. DNA lengths of multiples were determined from the relative mobilities of the centres of the bands. The DNA repeat length (numbers in parentheses) was calculated by dividing the lengths of each multiple by the number of the band

We are now examining the proteins associated with the nucleosomes and isolating the two monomers using salt precipitation and two dimensional electrophoresis.

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