BIOCHEMICAL EVIDENCE FOR A DISTINCTIVE CHROMATIN STRUCTURE IN NUCLEOLI OF *DICTYOSTELIUM*

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1. Introduction

The nucleosome is a ubiquitous unit of eukaryote chromatin [1–4]. The nucleosome core particles consist of 8 histone molecules and ~140 basepairs of DNA [1–4]. The length of the spacer DNA between nucleosomes is more variable, differing between species [5,6] and cell types in a particular tissue [7], while a natural homogeneity may exist within a given cell [8,9]. Transcribed and non-transcribed DNA sequences have been found organized in nucleosomes with the same repeat length [10], although the nucleosomes of potentially active genes do have a changed conformation [11,12]. The function of nucleosome heterogeneity is not known.

Estimates suggest that up to 15% of the eukaryote genome may not be packaged into nucleosomes [3]. Electron microscopy indicates that ribosomal RNA genes involved in transcription lack nucleosome structure [13–16]. Nevertheless, nuclease digestion shows ribosomal RNA genes do have a repeating structure, although whether this applies to both active and inactive genes is not known [10,17–21]. Lilley [3] notes that ribosomal RNA genes may have an exceptional nucleosome structure since they are generally 'super-active'.

The genome of *Dictyostelium discoideum* is only 11-times the size of the *Escherichia coli* genome [22] and the organism is widely used to study differentiation [23]. The morphological changes occurring during the life cycle are accompanied by modulation of gene activity [24].

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We have shown that *D. discoideum* DNA is packaged into nucleosomes [25] and these have been identified in the electron microscope [26]. We were interested to discover whether differences existed between the chromatin structure of ribosomal and non-ribosomal genes. *D. discoideum* ribosomal DNA consists of non-chromosomal palindromic dimers (minichromosomes) [27]. About 180 homologous copies of ribosomal DNA, comprising 17% of the nuclear DNA, are present per haploid genome [28,29].

Here we describe a method for isolating nucleoli and, using nuclease digestion, demonstrate differences in the chromatin structure of nuclei and nucleoli.

2. Materials and methods

D. discoideum cells (strain Ax-3) were cultured and nuclei isolated as in [30,31].

We initially attempted to isolate nucleoli using the method in [32] which involves sonication of nuclei. The method proved unsatisfactory, either resulting in chromatin associated with clumps of nucleoli or fragmenting the nucleoli. The French Press gave similar results.

The method we developed to isolate nucleoli involved, firstly, the production of crude nuclei. Cells were suspended in 1 vol. dist. water and 2 vol. cold SF-medium (0.5 M sorbitol, 2.5% Ficoll, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.5)) added. The final cell concentration was 4–5 × 10^8/ml. Triton X-100 was added to give 0.1% (v/v) final conc. and the suspension stirred for 5–10 min at 18°C. Intact cells were removed by centrifugation at

350 × *g* for 2 min. The lysate was homogenized (max. speed) for 50 s in an Omnimixer 17220 (Sorvall). It was immediately diluted with 1 vol. 20 mM Tris–HCl, 4 mM $CaCl_2$ (pH 7.5) and centrifuged at 15 000 × *g* for 15 min. The sediment, containing mainly nucleoli, was resuspended in 10 mM Tris–HCl, 2 mM $CaCl_2$ (pH 7.5) and centrifuged through a sucrose step gradient at 100 000 × *g* for 30 min. The sucrose concentrations were 1.5 M and 2.2 M in resuspension buffer. The sediment contained purified nucleoli.

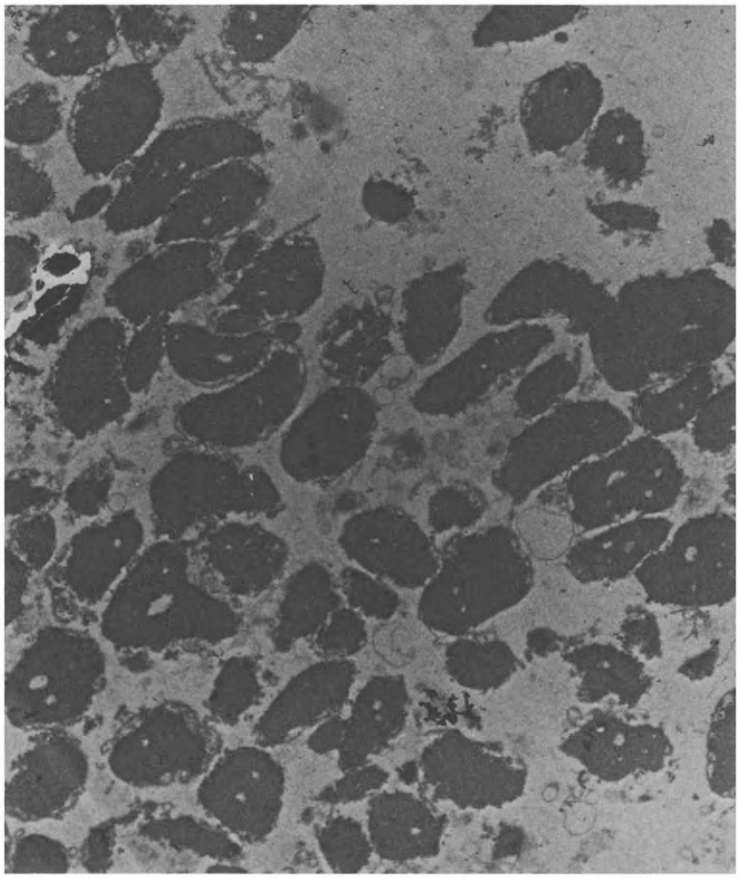
We also attempted to isolate nucleoli from purified nuclei using the same method. However, nucleoli obtained were aggregated and contaminated with non-nucleolar chromatin. This occurs because nuclei shrink during purification and fail to reach their original volume when reswollen in SF medium. Such partially shrunken nuclei are less efficiently broken during homogenization.

Nuclei (~3×10^9/ml) or nucleoli (~10^{10}/ml) were incubated with 150 and 75 units micrococcal nuclease (Worthington) per ml, respectively, as in [25]. DNA was extracted as before [25] except instead of adding Sarkosyl to the samples they were incubated with 100 μg/ml proteinase K (Boehringer) and 0.4% SDS for 3 h at 37°C DNA fragments were separated on acrylamide–agarose gels according to [5].

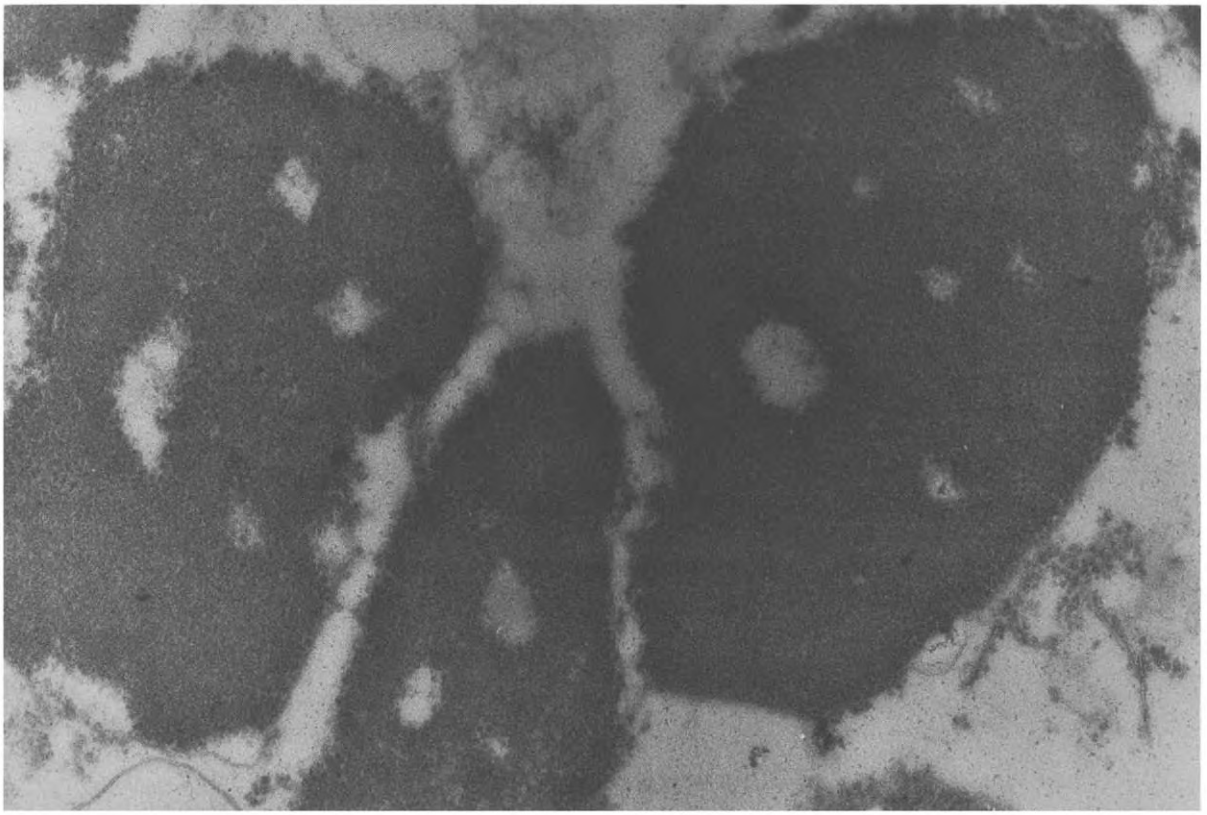
3. Results

Electron micrographs of the purified nucleoli are shown in fig.1.

When nucleoli were incubated with micrococcal nuclease characteristic patterns of DNA fragments were obtained (fig.3a,c). As with nuclei (fig.2b,c; fig.3b), two bands were present in the monosome



a



b

Fig.1. Preparations of purified nucleoli. (a) ×7500. (b) ×46 500.

region of the gel. On 5.5% polyacrylamide–agarose gels the lower band was seen to consist of two bands (fig.2b,c,e). The sizes of the monosomal bands were 168–175 (in some cases up to 185), 146–152 and 135 basepairs. Longer digestion resulted in cleavage of sites within the nucleosome core, producing a series of smaller DNA pieces (fig.2e).

The multimers obtained from nuclei are broad bands (fig.3b) and sometimes appear to consist of a number of bands [25]. The nucleolar digests contain DNA fragments resembling the nuclei multimers in size (fig.3). However, the nucleolar bands are sharper and the dimer and trimer, for example, consist of two bands at the upper and lower size limits of their respective nuclear multimers (fig.3a,b). The most striking difference is the presence of intermediate bands in the nucleolar digests (figs.3,4). Hence, two bands are always found between the dimer and trimer. In nuclear digests these bands are also present (fig.3b). They are, however, very weak, indicating they do indeed represent a minor fraction of the nuclear DNA.

A broad but very faint band is found between the dimers and monomers (fig.3c, fig.4). A band (200–210 basepairs) has also been observed above the monosomes in some, but not all, experiments (fig.3a).

4. Discussion

We describe a method for isolating nucleoli from *D. discoideum* cells. The sonication method [32] was not a success in our hands, producing either highly contaminated nucleoli or destroying them completely.

The DNA associated with the nucleosomal core of both whole nuclei and nucleoli is ~135 basepairs in length. The fragments of 146–152 basepairs may be

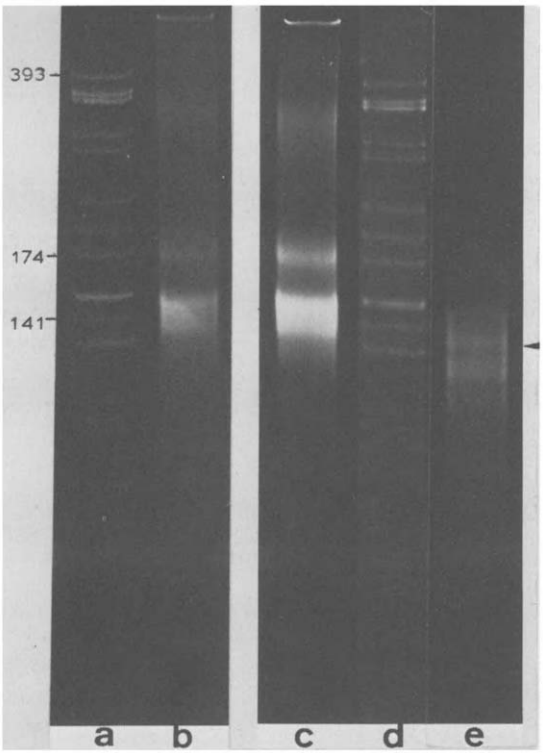


Fig.2. Polyacrylamide–agarose (5.5% and 0.5%) gel electrophoresis of micrococcal nuclease digests of nuclei (b,c,e). The arrow indicates the 135 basepair fragment. Gels were calibrated with a Hha I digest of plasmid pBR 322 DNA (a,d). The size (base pairs) of the marker fragments is: 393, 347, 337/332, 270, 259, 206, 190, 174, 153/152/151, 141, 132/131, 109, 104, 100.

derived from nucleosomes carrying histone H1. The 10–15 basepairs adjacent to the 135 basepairs of the core may be the site of strong interaction with histone H1 [33–35]. The largest monomers, which presumably include the linker DNA, consisted of 168–185 basepairs. Hence, the linker varies from ~30–45 basepairs in length. The value of 135 basepairs for the core is ~10 basepairs lower than for higher eukaryotes where 146 ± 3 has been reported [36]. A less likely possibility is that the 135 basepair fragment results from core digestion. The core would then be 146 basepairs when lacking H1 and 152 basepairs when H1 is present.

Our results differ from the report [26] that *D. discoideum* nucleosome contains 187 basepairs including a 137 basepair core.

Micrococcal nuclease digestion of nucleoli produces a consistent pattern of DNA fragments which is markedly different from the pattern obtained with whole nuclei. We have attempted to fit the fragment sizes into a model based on current ideas of chromatin structure. If all the fragments found are to be included we arrive at 4 different nucleosomal sizes (table 1). Nuclease splitting must also occur within the nucleosomal core, producing two halves of approximately equal size.

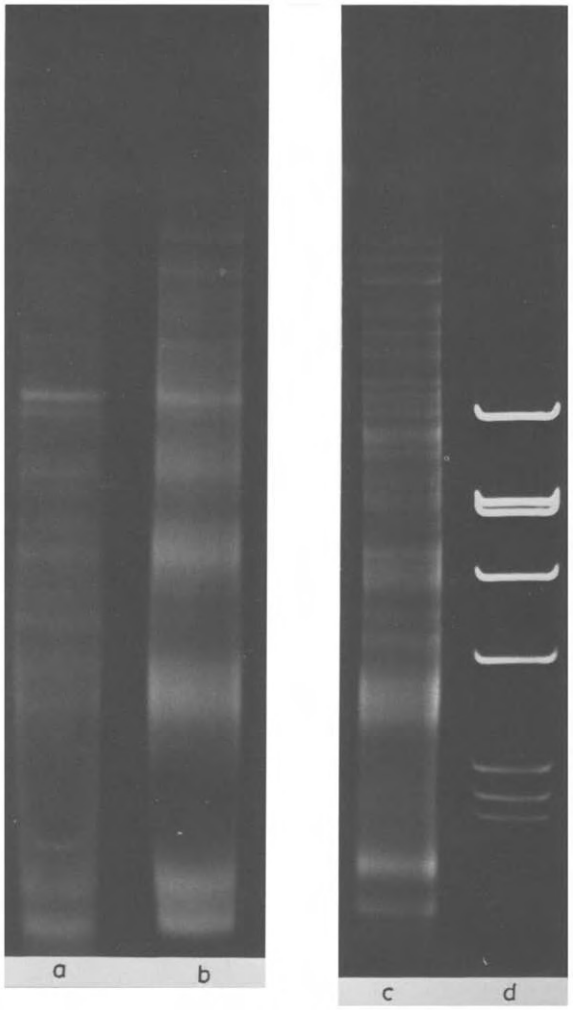


Fig.3. Polyacrylamide–agarose (2.0% and 0.5%) gel electrophoresis of micrococcal nuclease digests of nuclei (b) and nucleoli (a,c). Gels were calibrated with an *Alu* digest of pBR 322 DNA (d). The size (base pairs) of the marker fragments is: 910, 659, 655, 521, 403, 281, 257, 224.

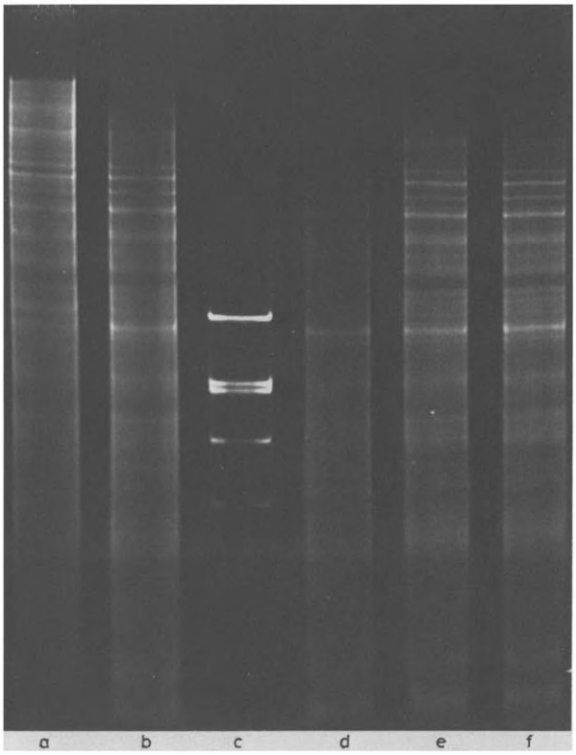


Fig.4. Gel system as in fig.3. Nucleoli were incubated with micrococcal nuclease (see section 2) for (a) 2 min, (b,e,f) 4 min or (d) 8 min. (c) *Alu* digest of pBR 322 DNA.

The simplest model would include only the 168 and 180 basepair nucleosomes. However, this would not account for three major bands (568, 780–800 and 950 base pairs). On the other hand, these fragments may be derived from regions lacking the 'typical' nucleosomal structure. Certainly, in most experiments no monosomes corresponding to the 210 and 228 basepair nucleosomes were detected. (Occasionally a 200–210 basepair band was present.) One could argue, however, that the linker regions of these monomers are exceptionally susceptible to digestion.

No sharp bands corresponding to 1.5 nucleosome fragments were found. However, a faint and broad band was present between monomer and dimer regions, suggesting the half-nucleosome is rapidly digested. Half-nucleosomes associated with two or more nucleosomes may retain their configuration and resistence to digestion.

The results suggest that nucleosomes of nucleoli possess a distinctive structure. Their apparent susceptibility to splitting may reflect a particular protein composition (e.g., histone modifications, non-histone proteins) of individual nucleosomes or packing of nucleosomal chains. We have found that deoxyribonucleoprotein particles from nucleoli lose many of their core proteins during gel electrophoresis,

Table 1
The lengths of DNA fragments obtained following micrococcal digestion of nucleoli fitted into a model postulating four different nucleosome sizes

Multiple	Postulated mononucleosome size (basepairs)							
	168		180		210		228	
	Predicted	Found	Predicted	Found	Predicted	Found	Predicted	Found
Dimer	336	330	360	365	420	420–440	456	450–460
2.5	420	420–440	450	450–460	530	500–535	570	568
Trimer	504	500–535	540	500–535	630	623	684	685
3.5	588	582	630	623	740[a]	720[a]	798	780–800
Tetramer	672	670	720	720	840[a]	840–850	912	910
4.5	756	–	810[a]	780–800[a]	945	950		
Pentamer	840	840–850	900	910				

[a] poor correspondence

whereas particles from non-nucleolar chromatin do not (unpublished results). The results give no indication of whether the distinctive nucleosomal structure is associated with active or inactive genes. We are using electron microscopy to examine the nucleosomal fibres.

The bands of nucleolar DNA are relatively narrow when compared with DNA fragments from whole nuclei. Random sliding of nucleosomal cores is probably not occurring. Various tandem arrangements of different sized nucleosomes would also not account for the fragment sizes observed. Nevertheless, some of the digestion products may reflect a novel chromatin structure with histones, for example, lying along the DNA strand. Different regions of the genome may have different structures. We are using DNA–ribosomal RNA hybridization to check this possibility.

In conclusion, nuclease digestion indicates that nucleolar chromatin possesses a unique structure. Digestion produces a consistent pattern of DNA fragments which give sharper bands than whole nuclear digests. Most striking are the bands appearing between nucleosome multimers (e.g., dimers and trimers). Analysis of DNA fragments suggests at least 2 and possibly 4 different nucleosome sizes are present and that the nucleosome core may be split by the nuclease. This implies a unique association of proteins with nucleolar DNA, reflecting perhaps the presence of specific histone and non-histone proteins which modify the structure of individual nucleosomes or the packing of nucleosomal chains. The core-splitting may be related to the 'super-activity' of ribosomal RNA genes [3].

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