DNA REPEAT LENGTH OF CHROMATIN FROM THE UNICELLULAR ALGA *OLISTHODISCUS LUTEUS*

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1. Introduction

A rather consistent model for the subunit structure of eukaryotic chromatin has emerged during the past several years. The basic repeat unit, the nucleosome, consists of a core region plus a linker region connecting adjacent core units. The earliest work on nucleosome structure proposed an invariable repeat unit of ~200 base pairs [1,2], but later work revealed a small variability amoung higher eukaryotes and a difference between higher and lower eukaryotes [3]. Depending on the organism, the amount of DNA within the repeat unit ranges between 150 and 200 base pairs. Repeatedly, the nucleosome core has been found to contain 140 base pairs of DNA plus an octamer of histone molecules. However, the linker region has been reported to contain between 25 and 80 base pairs, thus providing the variability of the length of DNA found in the nucleosome repeat unit [4–12]. While the repeat unit in the higher eukaryotes varies between 170–200 base pairs [3], the repeat unit reported for the fungi ranges between 150–170 base pairs, establishing a much shorter length in the lower eukaryotes [4–12]. Although no data concerning the chromatin repeat size in eukaryotic algae has been reported to date, it might be expected that, as with the fungi, a shorter linker would be demonstrated. We report here that the linker length in the unicellular alga *Olisthodiscus luteus* is larger than that found in higher eukaryotes.

2. Materials and methods

Cultures were maintained in 0–3 medium [13] at 22°C under a 12 h light–12 h dark regime, and nuclei were isolated as in [14]. Rat liver nuclei and chick liver nuclei were isolated as in [15]. All nuclear pellets were washed in a 10 mM Pipes buffer (pH 7.5) containing 5 mM $MgCl_2$, 5 mM $CaCl_2$ and 1.0 M sucrose and resuspended in 1 ml fresh buffer. Staphylococcal nuclease (Worthington) 20 μl (200 units) were added to each nuclear suspension. The suspensions were then incubated at 37°C for 15 min. Samples were taken at various intervals during limit digestion of *Olisthodiscus* chromatin. The reaction was stopped by the addition of an equal volume of 1 M perchloric acid containing 1 M NaCl and 20 mM ethylenediaminetetra-acetic acid (EDTA). Samples were clarified by centrifugation in a tabletop clinical centrifuge and the supernatants were transferred to clean test tubes. RNAase (Sigma) 10 λ (100 units) was added to each tube and the tubes were incubated at 37°C for 1 h. A lysing buffer containing 10 mM NaCl, 5 mM EDTA, 1 mg/ml Pronase (Worthington), and 2% (w/v) sarcosyl *n*-lauryl sarcosine (Sigma) was then added to each tube and incubation continued for 5 h. The aqueous mixture was then extracted 3 times using equal volumes of chloroform:isoamyl alcohol (24:1). The DNA was precipitated with 2 vol. cold 95% ethanol at –5°C overnight, pelleted by centrifugation and resuspended in 100 mM Tris (pH 7.5), 1 mM EDTA. The A_{260} of each suspension was read to determine the amount of DNA recovered. The samples were prepared for electrophoresis by addition of 40 mM Tris (pH 7.5), 30 mM NaH_3PO_4, 5 mM EDTA and 20% glycerol (w/v)/0.01% bromophenol blue solution.

3. Results and discussion

Isolated nuclei from *O. luteus*, rat liver and chick liver were incubated with staphylococcal nuclease

and the acid-soluble fractions recovered. The DNA recovered from these fractions was then electrophoresed on 6% polyacrylamide gels (fig.1). *Hae*III and *Hin*dIII digests of PM2 DNA were used as molecular weight markers.

Fig.1 does not show digestion down to the nucleosome core for any of the three organisms; however, examination of the multimer bands reveals that those of *Olisthodiscus* are consistently higher than their counterparts for rat or chick liver.

The lengths of these DNA fragments was calculated by least squares analysis of the logarithm of the base pairs versus their migration distance using the *Hae*III and *Hin*dIII fragmented DNA of PM2 (fig.2). The calculated lengths of the various DNA multimers reiterates the trend seen in fig.1. The DNA repeat length for each organism was determined by dividing the fragment lengths by the corresponding band number (fig.3). The basic repeat size was found to be 185 base pairs for rat liver and 195 base pairs for chick liver nuclei. These values are consistent with the 200 base pair average repeat size seen in higher eukaryotes. The repeat size for *Olisthodiscus* chromatin, however, was found to be 220 base pairs, which is much higher than one would expect for a lower eukaryote [1–9].

Although the core unit of 140 base pairs has been demonstrated consistently for all eukaryotic chromatin, the linker region has shown great variability. Members of the fungi studied thus far have shown an overall pattern of shorter linker regions (15–30 base pairs). Limit digestion of chromatin from *Olisthodiscus* also reveals a core unit of 140 base pairs (fig.4). As the time of digestion progressed, bands became more visible in the trimer, dimer and monomer bands. After 39 min digestion, a smear can be seen below the monomer band, which probably represents digestion of the nucleosome core.

The 140 base pair core and 220 base pair repeat size gives a linker length of 80 base pairs, which is nearly twice that found in the fungi. It is noteworthy that long repeat sizes have also been reported for the macronucleus of *Tetrahymena* [16,17] and the micronucleus, macronuclear anlagen and macronucleus of *Stylonychia* [18]. As in *Olisthodiscus*, these longer repeat sizes are due to longer linker regions. It has been suggested that variations in linker length between higher and lower eukaryotes may be a result of differences in the lysine-rich histones H1, H2A and H2B, and that the constancy of the 140 base-pair

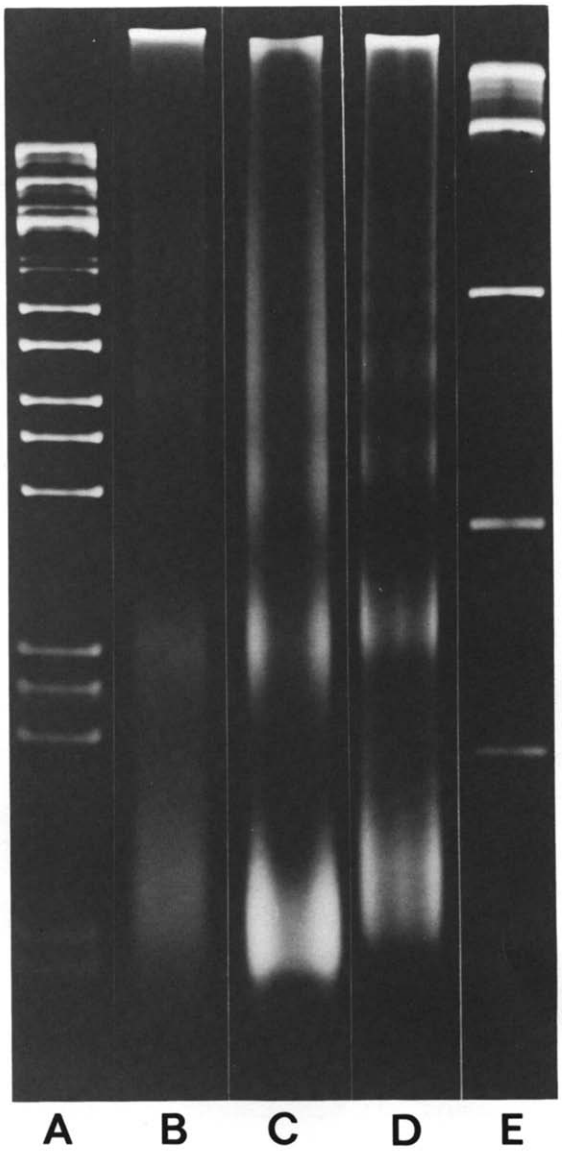


Fig.1. Electrophoresis of nuclease digestion products. Nuclei from rat liver, chick liver, and *Olisthodiscus luteus* were incubated with staphylococcal nuclease, and the DNA was prepared as in section 2. The samples were then electrophoresed in a 6% acrylamide, 0.16% bis gel at 25 V for 12 h and visualized by staining with ethidium bromide (1 μg/ml). The gels were photographed using a UV filter (Soligar) in conjunction with an orange gel filter 23A (Kodak). *Hae*III and *Hin*dIII digests of PM2 DNA were utilized as molecular weight standards. (a) *Hae*III digest of PM2. (b) Rat liver DNA fragments. (c) Chick liver DNA fragments. (d) *O. luteus* DNA fragments. (e) *Hin*dIII digest of PM2.

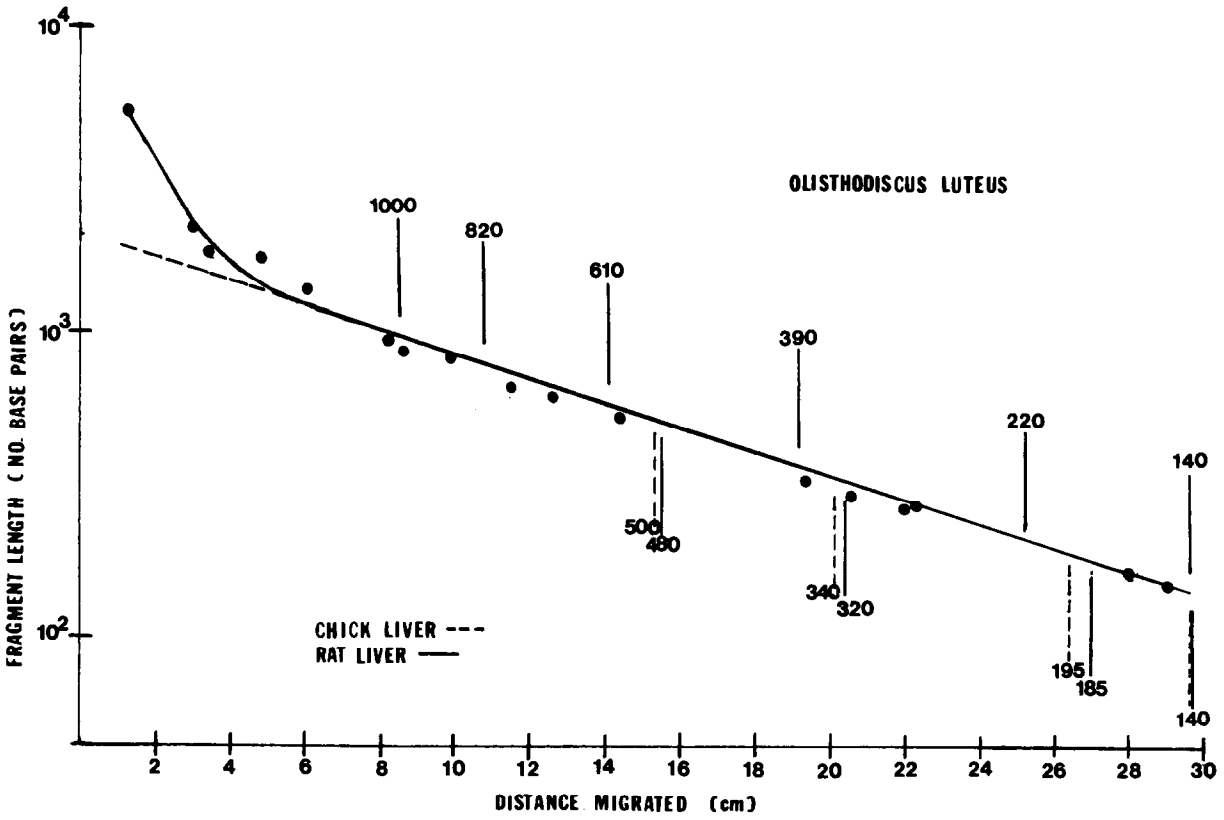
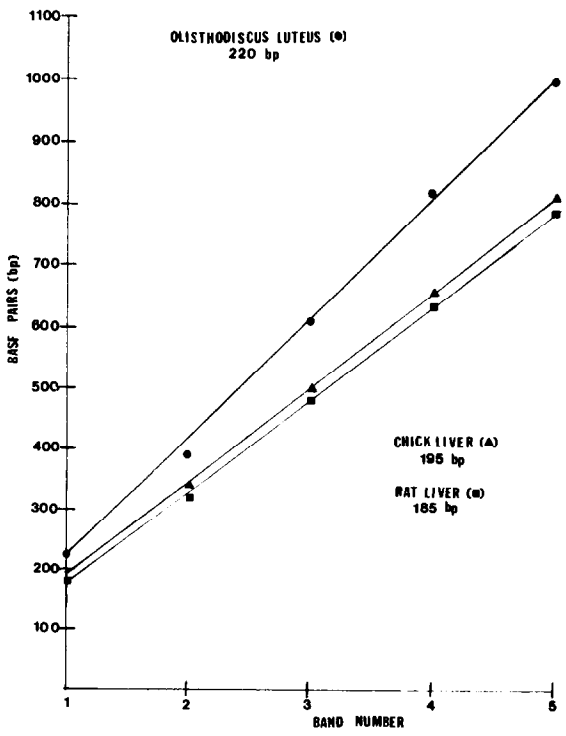


Fig.2. Sizing of DNA fragments. DNA fragments from staphylococcal nuclease digest of *Olisthodiscus*, rat and chick liver nuclei were electrophoresed with calibrated *Hae*III and *Hin*dIII restriction endonuclease fragments from phage PM2 DNA. Closed circles indicate the position of the PM2 DNA fragments. Values above the calibration curve are the mean lengths for *Olisthodiscus* fragments. Solid lines below the calibration curve are the mean lengths for rat liver DNA while dashed lines are the mean lengths for chick liver DNA. The calibration curve is representative of 3 nearly identical curves constructed from least squares analysis using DNA fragments of 1.4×10^3 base pairs and smaller.



core reflects the extreme conservation of the arginine-rich histones H3 and H4 (discussed [9]). Although *Olisthodiscus* histones have not yet been characterized, chromatin from this primitive eukaryote is organized into beaded strands [14], but does not contain a full complement of histones [19]. Of the 4 histones that are detectable, only those corresponding to H3 and H4 have identical mobilities with calf thymus histones in both acidic–urea, and SDS-containing gels [19]. Irrespective of the reason for variations in repeat size, other groups of algae now need to be examined to see if a long repeat size is characteristic of algae in general, or unique to *Olisthodiscus*.

Fig.3. Determination of DNA repeat size. The lengths in base pairs of *Olisthodiscus*, rat liver and chick liver DNA fragments were determined from the calibration curve shown in fig.2. DNA repeat sizes were obtained by taking the slope of a plot of DNA fragment length (in base pairs) against multimer number. (●) *Olisthodiscus luteus;* (▲) chick; (■) rat.

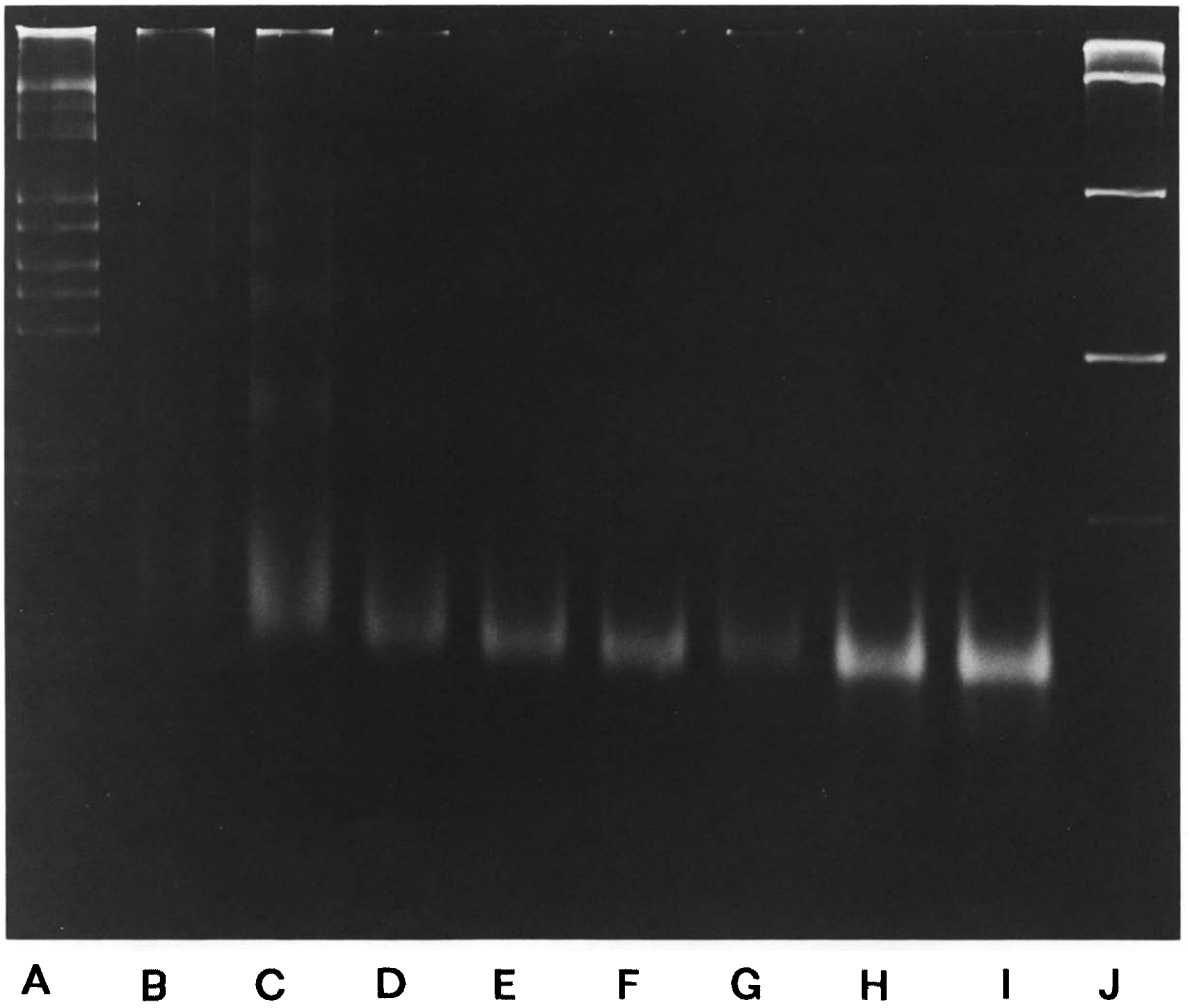


Fig.4. Limit digestion of olisthodiscus chromatin. Nuclei from *Olisthodiscus luteus* were incubated with staphylococcal nuclease and samples were taken at various time intervals. Electrophoresis was on a 6% polyacrylamide gel. (a) *Hind*III digest of PM2 DNA. (b–i) digests of *Olisthodiscus* chromatin (min): (b) 3; (c) 9; (d) 15; (e) 21; (f) 27; (g) 33; (h) 39; (i) 45. (j) *Hae*III digest of PM2 DNA.

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