Accessibility of histone H4 gene of *Physarum polycephalum* to DNase I during the cell cycle

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DNase I was used as a probe to detect conformational changes of the H4 histone gene of *Physarum polycephalum* during the cell cycle. The degradation of histone genes was followed by gel electrophoresis and hybridization with a probe for the H4 histone gene. It was found that even during mitosis when chromatin is condensed into chromosomes, the histone genes are preferentially degraded by DNase I. The histone genes retain a characteristic structure which is recognized by DNase I during all stages of the cell cycle and thus independently of the biosynthesis of histones.

Physarum *Histone gene* *DNase I* *Cell cycle* *Chromatin* *Southern blot*

1. INTRODUCTION

The slime mold *Physarum polycephalum* has become an important model system for investigating the cell cycle in eukaryotes because of the extreme natural synchrony of the many nuclei (10^8–10^9) of the macroplasmodia obtained in surface culture. We have shown [1] significant differences in the structure of chromatin in metaphase and interphase (S or G_2). Moreover, the structure of newly replicated chromatin in S phase differs from the bulk in that it is more easily degraded by either staphylococcal nuclease or by DNase I.

In a wide variety of organisms and tissues transcriptionally or potentially active genes exist in an altered conformation in nuclear chromatin and are preferentially degraded by DNase I [2–7]. It was our purpose to determine whether the apparent conformational change of chromatin that accompanies gene activation in higher eukaryotes also occurs in *Physarum* and whether the sensitivity to DNase I can be observed during all stages of the cell cycle.

Here, we have used DNase I as a probe to examine the nuclease sensitivity of the histone H4 gene at different stages of the cell cycle. The degradation of histone genes was followed by gel electrophoresis and hybridization with a probe for the H4 histone gene. The results show that the histone genes are preferentially degraded by DNase I regardless of the stage of the cell cycle and thus independently of the biosynthesis of histones.

2. MATERIALS AND METHODS

Synchronous macroplasmodia were grown on filter papers supported by glass beads according to [8]. The cell cycle stage was determined by phase-contrast microscopy. At each time point the rate of DNA synthesis was monitored by labeling the macroplasmodia with [^{3}H]thymidine (300 μCi/ml) for 10 min, precipitating the material with trichloro-acetic acid and measuring its activity in cpm.

The rate of protein synthesis was monitored by labeling cultures for 10 min with a mixture of [^{14}C]arginine (2.5 μCi/ml). For each time point total cellular protein was recovered as follows: 1/8th of a macroplasmodium was homogenized in 10 ml 5% trichloroacetic acid, 48% water, 47% acetone, left 15 min at 0°C and 15 min at 20°C, centrifuged and the pellet washed in acetone and dried. The proteins were hydrolysed with NaOH and the amount of ^{14}C-labeled lysine and arginine

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incorporated was then determined. Nuclear proteins were recovered as above except that nuclei were prepared prior to the homogenization in trichloroacetic acid, water and acetone.

Physarum nuclei were purified as in [1]. For the DNase I digestion the nuclei were dispersed in 0.25 M sucrose, 10 mM $MgCl_2$, 1 mM $PhMeSO_2F$, 10 mM Tris–HCl (pH 7.4) and incubated for various times with 2 units/ml of DNase I. The reaction was stopped by adding EDTA to 20 mM final conc.

The resistant DNA was deproteinized by incubation with proteinase K (100 μg/ml) at 37°C for 1 h in presence of 1% SDS. DNA was extracted by two treatments with 1 vol. phenol and two treatments with chloroform/isoamyl alcohol (9/1). The DNA in the aqueous phase was precipitated with 2 vol. ethanol at −20°C overnight. The digested DNA was fractionated by agarose gel electrophoresis using a horizontal slab gel apparatus. DNA was transferred from agarose gels to NEN GeneScreen filters as in [9]. DBM paper for Dot-blotting was prepared as in [10]. The dot-blotting and hybridization with the DBM paper were performed as in [11]. Hybridization with the radioactive probe was carried out at 65–68°C in 3 × SET, 10 × Denhardt's solution, 0.5 M NaCl, 0.1% SDS (20 × SET is 3 M NaCl, 0.6 M Tris–HCl (pH 7.6), 0.04 M EDTA; 50 × Denhardt's solution is 1% Ficoll 400, 1% PVP 40, 1% BSA [12].

Washing after hybridization was carried out at 65°C as follows:

(a) 1 × 250 ml of a solution containing 0.5 M NaCl, 3 × SET, 0.1% SDS, 0.1% $NaPP_i$, 5 × Denhardt's solution;
(b) 1 × 250 ml of 3 × SET, 0.1% SDS, 0.1% $NaPP_i$;
(c) 1 × 250 ml of 2 × SET, 0.1% SDS, 0.1% $NaPP_i$;
(d) 1 × 250 ml of 1 × SET, 0.1% SDS, 0.1% $NaPP_i$.

Nick translation was performed using a nick translation kit from BRL and carried out according to the manufacturer's conditions. The histone probe used was the H4 histone gene isolated by *Eco*RI digestion from the plasmid pHae 181 [13] (a kind gift of Dr Max L. Birnstiel, University of Zürich).

A fragment of the central spacer region of the extrachromosomal ribosomal DNA was used as a probe for non-transcribed gene and was isolated by *Hpa*I digestion of the plasmid pPHR 115 (a kind gift of Dr R. Braun, University of Bern). The *Hpa*I fragment is 410 basepairs and situated at 20.4 kilobases from each end of the palindromic rDNA molecule.

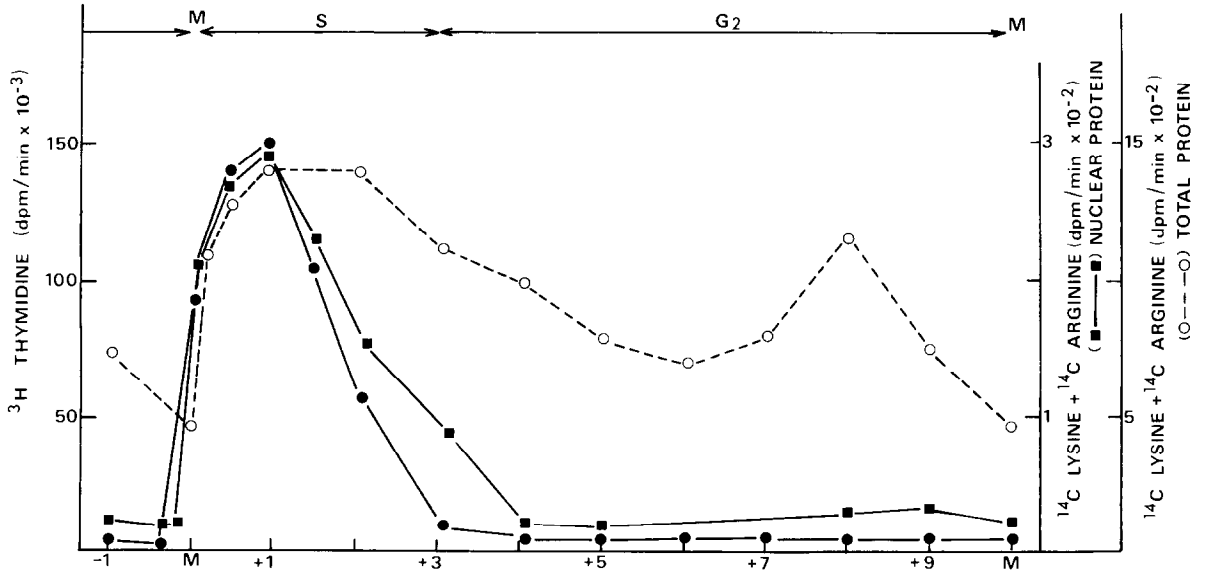

Fig. 1. Synthesis of DNA, total cellular proteins and nuclear proteins during the cell cycle of *Physarum*.

3. RESULTS

3.1. *Synthesis of DNA, total proteins and nuclear proteins during the cell cycle*

We have measured the rate of DNA synthesis, total cellular proteins and nuclear proteins at various times after mitosis. In *Physarum* macroplasmodia there is no G_1 period and interphase begins with the S phase which lasts ~3 h in standard conditions of growth. The G_2 phase which follows lasts ~6 h. To simultaneously monitor the rate of protein and DNA synthesis the cultures were labeled with a mixture of [^{3}H]thymidine, [^{14}C]lysine and [^{14}C]arginine. In agreement with previous reports (review [14]), the onset of DNA synthesis occurs immediately after the completion of mitosis (fig. 1), the incorporation of [^{3}H]thymidine increases rapidly, is maintained at a high level during most of S phase and decreases rapidly at its end. The rate of nuclear protein synthesis, measured in isolated nuclei, follows closely the rate of DNA synthesis. Preliminary results (submitted) indicate that the same direct correlation between the rate of DNA and histone synthesis is found during the S phase. This is consistent with reports that in several other cells histone and DNA synthesis are tightly coupled during the cell cycle [15,16]. In *Physarum* the ratio histone/DNA is 1 : 1 during all stages of the cell cycle [17] also suggesting that histones are synthesized at the same time as DNA during S phase. We have also measured the rate of total cellular protein synthesis as a control for these experiments. In contrast to DNA and nuclear protein synthesis we find that the rate of total protein synthesis shows a biphasic pattern. The minima in the pattern of incorporation occur at mitosis and in mid-interphase, one maximum coincides with the maximum of DNA synthesis and another occurs in G_2 phase.

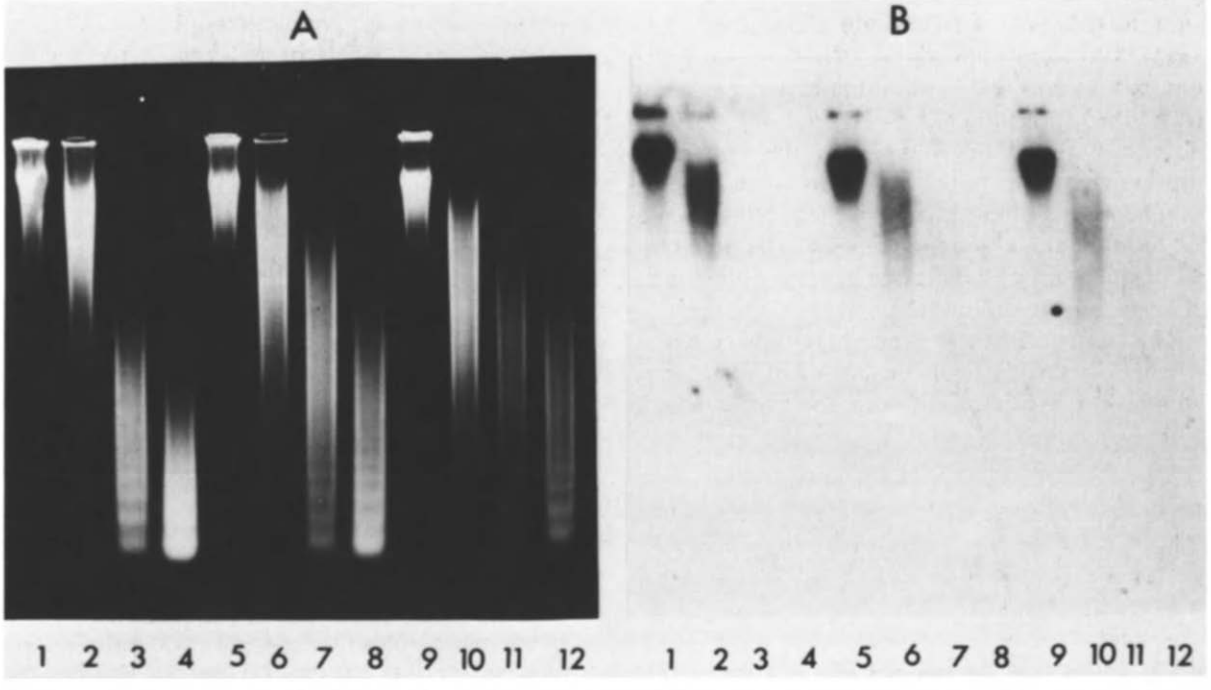

Fig. 2. DNase I digestion of *Physarum* nuclei. The nuclei were digested to several degrees at various stages of the cell cycle; slot no. 1–4, mitosis 1%, 4.5%, 15% and 21% acid-soluble DNA products; slot 5–8, S phase 1.2%, 4.9%, 12%, 20% acid-soluble DNA products; slot 9–12, G_2 phase 0.9%, 5.9%, 11% and 14% acid-soluble DNA products. (A) Electrophoretic profile after ethidium bromide staining of the gel; (B) autoradiograph of the blot obtained after hybridization with the H4 histone probe.

3.2. *Sensitivity of* Physarum *genes to DNase I*

The evolutionary conservation of histone H4 coding sequences allows the detection of *Physarum* histone sequences by cross-species hybridization with a probe derived from sea urchin. The heterologous sea urchin probe was a kind gift of Dr M. Birnstiel and contains the coding region of the sea urchin histone H4 between amino acid codon 3 and amino acid codon 94 [13]. To investigate the sensitivity of histone H4 gene to DNase I, synchronous surface cultures of *Physarum* were grown and nuclei from S phase (mitosis + 1 h), G_2 phase (mitosis + 5 h) and metaphase were isolated. The respective nuclear preparations were digested to several degrees (4.5–21% acid solubility) with DNase I and the resistant DNA purified and analysed on 1% agarose gels (fig. 2A): DNase I decreases the size of the DNA fragments quickly; however, when 20–21% of the DNA has become acid-soluble the size of the DNA fragments remains between 0.2–1.2 kilobases. The gels were then blotted on nitrocellulose filters, the filters hybridized to the radioactive H4 probe and autoradiographed. Alternatively aliquots of resistant DNA were spotted on DBM paper (dot-blotting), hybridized to the radioactive probe and autoradiographed (fig. 2B,3). When > 11–15% of the chromatin DNA is digested, the remaining resistant fragments fail to hybridize to the H4 probe. The results obtained by Southern blotting and dot blotting are in good agreement. The preferential degradation of the histone genes is observed at all stages of the cell cycle.

As a control for these experiments and to check whether the small size of the DNA fragments does not prevent hybridization with the probe, naked *Physarum* DNA was digested with DNase I to the same extent as the nuclear preparation. The resulting DNA was analysed by agarose gel electrophoresis (fig. 4A) and dot blotting (fig. 4B). In contrast to the result obtained with the nuclei, the H4 probe hybridizes with the resistant DNA fragments obtained for all levels of DNase I digestion. It appears that at the highest level of DNase I digestion hybridization is not as good as with high-M_r DNA but it is however significantly higher than the hybridization obtained with the DNA purified from nuclei digested to a similar extent. As a further control we have examined the DNase I sen-

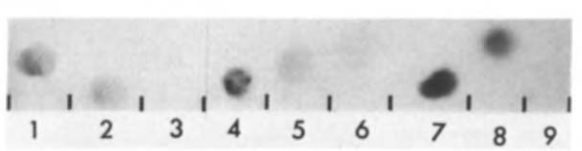

Fig. 3. Dot blots of the resistant DNA fragments after DNase I digestion of *Physarum* nuclei at various stages of the cell cycle. For each dot 5 μg of DNA was spotted on the DBM paper: (1–3) G_2 phase 5.9%, 7.7%, 14% acid-soluble DNA products; (4–6) S phase 4.9%, 12%, 15.7% acid-soluble DNA products; (7–9) mitosis 5.7%, 6.8%, 21% acid-soluble DNA products.

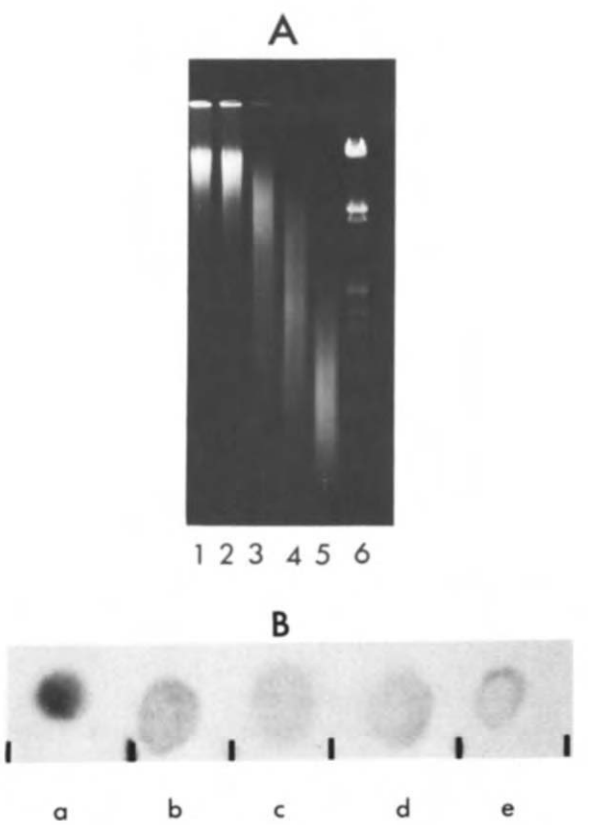

Fig. 4. DNase I digestion of *Physarum* DNA. (A) Electrophoretic profile of the resistant DNA fragments after ethidium bromide staining of the gel. M_r-Value markers are shown in slot 6 (*Hin*dIII + *Eco*RI digest of λ DNA, from top: 21.8, 5.24–5.05, 4.21, 3.41, 1.98, 1.90, 1.71, 1.35, 0.93, 0.84, 0.56 kilobases). (B) Dot blots of the resistant DNA fragments. Dots a–e correspond to the DNA fragments analysed in slots 1–5 of the agarose gel. For each dot 5 μg DNA was spotted on the DBM paper.

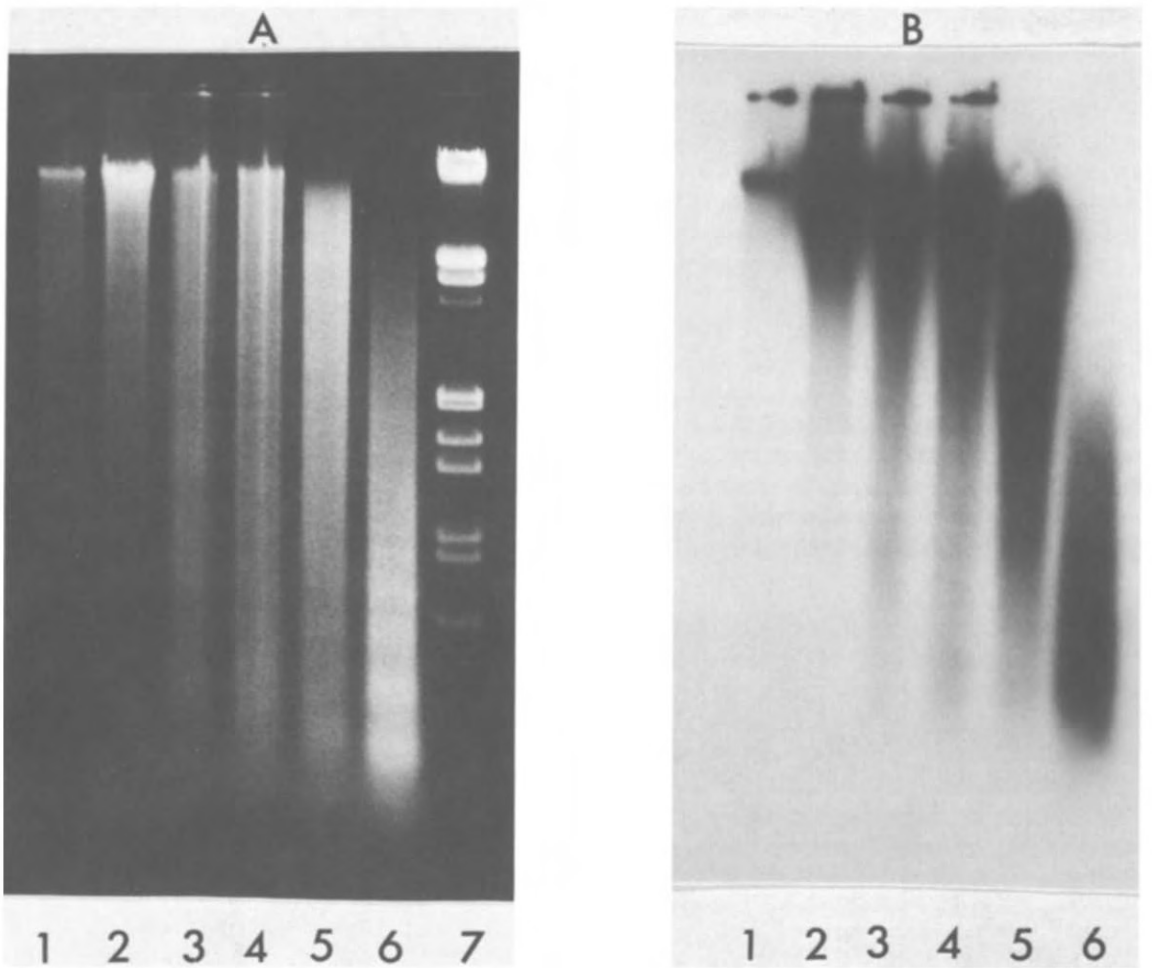

Fig. 5. Digestion of *Physarum microplasmodia* nuclei with DNase I. (A) Electrophoretic profile after ethidium bromide staining of the gel. M_r markers are shown in slot 7: (*Hin*dIII + *Eco*RI digest of λ DNA, from top: 21.8, 5.24–5.05, 4.21, 3.41, 1.98, 1.90, 1.71, 1.35, 0.93, 0.84, 0.56 kilobases). (B) Autoradiograph of the blot obtained after hybridization with the *Hpa*I fragment of the central spacer region of the extrachromosomal ribosomal DNA.

sitivity of a DNA sequence situated in the central spacer region of the extrachromosomal ribosomal DNA and which is not transcribed. The non-transcribed sequences are not preferentially digested by DNase I (fig. 5A,B).

4. CONCLUSION

In many eukaryotes histone biosynthesis and DNA replication are temporally regulated and occur during the S phase. Several experiments suggest that this is also true in *Physarum*. Further work is needed to elucidate at what time during the cell cycle the mRNA for the histones is synthesized and whether the transcription of histone genes is restricted to the S phase as in some of the other eukaryotic cells studied [18–20]. However, it is safe to say that transcription of histone genes into mRNA is reduced to a very low level during mitosis, since [21] precursor incorporation decreases to an undetectable level as *Physarum* passes from prophase to metaphase. Here, we have used DNase I to detect conformational changes of the H4 histone gene during the cell cycle. We find

that even during mitosis when chromatin is condensed into chromosomes and most active genes are silent, the H4 histone genes retain a characteristic structure which is recognized by DNase I. This result agrees with [4] that in the oviduct the degree of sensitivity to DNase I does not depend upon the rate of transcription. In [22] the same conclusion that the *Physarum* extrachromosomal ribosomal genes are preferentially degraded by DNase I irrespectively of their actual activity, was reached. The entire genome of *Physarum* is not equally sensitive to DNase I as in yeast [23] since we show that non-transcribed sequences are not preferentially digested by DNase I. It would now be interesting to study the behavior of other *Physarum* active genes during the cell cycle and also to determine whether active genes can be shut off when *Physarum* is induced to differentiate into dormant cells (spores or spherules).

ACKNOWLEDGEMENT

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