Eur. J. Biochem. 44, 407–410 (1974)

Reassociation Kinetics of Nuclear DNA from *Physarum polycephalum*

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(Received November 16, 1973/February 18, 1974)

Nuclear DNA of *Physarum* is made up of 45% repeated ($c_0 t_{1/2} = 0.07-0.7$ mol · l^{-1} · s) and 55% non-repeated base sequences ($c_0 t_{1/2} = 500-1100$ mol · l^{-1} · s) and has a complexity of 130 times the *Escherichia coli* genome. DNA replicated early in the S phase of the cell cycle, contains few repeated base sequences (below 10%).

Reassociation kinetics of denatured DNA has been used as a tool to distinguish repeated from non-repeated base sequences in DNA from eucaryotes [1]. Further analyses of reassociation data allow the characterization of genomes from different organisms in terms of relative amount of repetitive DNA, repetition frequency and complexity [1–4].

In this paper we describe the genome of *Physarum polycephalum* and some experiments which indicate fewer repeated sequences in DNA which is replicated early during a naturally synchronous S phase.

MATERIALS AND METHODS

Cultures

Physarum was grown in a semi-defined medium as described previously [5]. Microplasmodia were kept as a shaken suspension and transferred serially every 2–3 days. Microplasmodia from exponential growth phase were harvested by centrifugation ($50 \times g$, 1 min), pipetted onto filters of 8-cm diameter (Schleicher & Schüll no. 576 or millipore), supported by a stainless steel grid and allowed to fuse into macroplasmodia in which nuclei divide synchronously every 8–10 h at 26 °C. Mitotic stages were accurately determined by phase-contrast microscopy. Mitosis was immediately followed by DNA replication (S phase), which lasted for about 3 h.

Preparation of DNA

Nuclei were isolated using published procedures [6]. Labelling of DNA was done with [^{3}H]thymidine: (200 μCi/ml medium, specific activity 17000 Ci/mmol, Amersham). DNA was isolated after published methods [7] and purified by CsCl gradient centrifugation. Main band DNA (1.700 g/ml) was dialysed against 0.1 standard saline citrate and sheared by sonication (Branson sonifier, 3 min at full intensity). Average molecular weight of the fragments was 3.5×10^5 of single-stranded DNA [8,9], hyperchromicity 38%, $T_m = 86.5$ °C. No DNA was released from hydroxyapatite in 0.12 M phosphate buffer at 62 °C.

Reassociation was calculated from $c_0 t$ values obtained by two common methods [1].

Ultraviolet Spectrophotometry

Samples of DNA (up to 400 μg/ml in standard saline citrate, 30% formamide, 0.1% sodium dodecylsulfate) were heated to 80 °C in a sealed quartz cuvette of 1-mm optical pathlength, quickly cooled to and maintained at 38 °C. Loss of hyperchromicity was measured at 260 nm in a Zeiss spectrophotometer.

Hydroxyapatite Chromatography

Samples of DNA (5 μg in 5–50 μl standard saline citrate) were sealed in capillary tubes, heated for 10 min at 100 °C and cooled to and maintained at 62 °C (or 38 °C in the presence of 30% formamide) for times up to several weeks. After incubation over 95% of the DNA were recovered from hydroxyapatite columns and 98% remained trichloroacetic-acid-precipitable. Relative amounts of single or double-stranded DNA (800–22000 counts × min^{-1} × μg DNA^{-1}) were computed from fractions eluted at 0.12 M and 0.35 M phosphate at 62 °C, respectively.

Abbreviations. Standard saline citrate, 0.15 M NaCl, 0.015 M sodium citrate pH 7; $c_0 t$, nucleotide concentration of annealing solutions (in mol/l) × time (in s) of incubation.

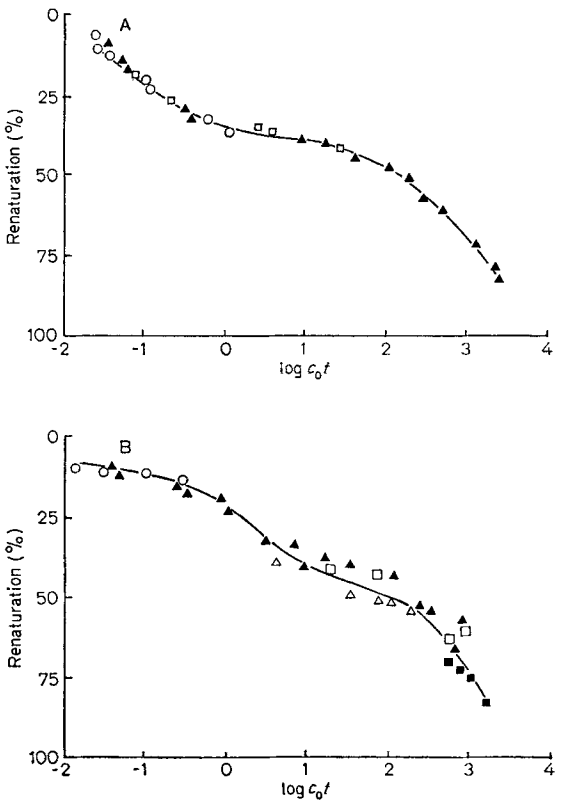

Fig. 1. *Renaturation of main band DNA from* Physarum. (A) Hyperchromicity was continually measured at 260 nm (0% reassociation corresponds to 38% hyperchromicity) of heat-denatured DNA). (○) 235 μg DNA/ml, (□) 270 μg/ml, (▲) 396 μg/ml. (B) For each point 5 μg DNA were analysed on a 0.5 ml hydroxyapatite column. (○) 100 μg DNA/ml, (△) 250 μg/ml, (▲) 500 μg/ml, (□) 900 μg/ml, (■) 1000 μg/ml

RESULTS AND DISCUSSION

Reassociation of Main Band DNA of Physarum

Renaturation of nuclear DNA was calculated from the loss of hyperchromicity (Fig. 1A). We observed a biphasic pattern with a plateau in the range of $c_0t = 1-100$ mol · l^{-1} · s. The repetitive fraction comprised 42% and a $c_0t_{1/2}$ of 0.07 mol · l^{-1} · s could be calculated [2]. The remaining 58% of nuclear DNA reassociated with a $c_0t_{1/2}$ of about 1100 mol · l^{-1} · s. The frequency of repetitive DNA sequences measured by this method was approximately 15000.

Reassociation measured by hydroxyapatite chromatography (Fig. 1B) also showed repeated and unique sequences separated by a plateau (c_0t = 10 to 100 mol · l^{-1} · s). Again 50—60% of the DNA renatured with a $c_0t_{1/2}$ of 500—1000 mol · l^{-1} · s. A mean $c_0t_{1/2}$ value of 0.7 mol · l^{-1} · s was calculated from several experiments for the repetitive DNA fraction and therefore the multiplicity measured by this method is approximately 1500.

A high percentage (about 50%) of repetitive DNA of *Physarum* with a $c_0t_{1/2}$ of about 1 mol · l^{-1} · s and a repetition frequency of about 2.000 had previously been observed [10] (and Evans, Cleveland, personal communication).

We calculated the proportions of repetitive and unique base sequences by dividing our data in two groups and drawing normalized c_0t curves for each group [2]. We then plotted a set of second-order curves to fit our experimental data. The $c_0t_{1/2}$ values for the repetitive fraction obtained from these calculations were significantly lower in the hyperchromicity recovery curves as compared with those obtained after chromatography on hydroxyapatite at 62 °C (0.07 *vs* 0.7). Such differences had been observed previously for the repetitive DNA of *Physarum* [11]. Therefore, we assumed a high degree of mismatching and complex formation among single-stranded DNA molecules causing a more rapid loss of hyperchromicity. The fraction of DNA retained on hydroxyapatite ($c_0t_{1/2}$ = 0.7 mol · l^{-1} · s, 0.12 M phosphate buffer, 62 °C) was unstable at high temperatures and eluted from the column by raising the temperature to 82 °C in steps of 4 °C in an almost linear fashion. Also, our experimental data did not fit perfectly the theoretical curve at c_0t values below 0.3 mol · l^{-1} · s, indicating that our repetitive DNA renatured faster (up to 10%) than expected. Since we obtained rather large pieces of DNA by sonication, we assumed that intramolecular heterogeneity also contributed to the thermal instability of our fast reannealing DNA fraction. Our calculation of the sequence length of 4×10^5 nucleotide pairs, based on a $c_0t_{1/2}$ value of 0.7 mol · l^{-1} · s, could only be taken as an estimate although it was similar to published data on intermediate repetitive base sequences of other lower eucaryotes [12].

In *Physarum*, a small highly repetitive fraction and two families of intermediate repetitive DNA molecules had previously been described [11]. A heavy satellite DNA (rich in rDNA) of 1—2% of the total DNA had been isolated [13] and the multiplicity of this DNA fraction agreed well with the number of ribosomal cistrons (1000—2000) detected in the nucleoli of *Physarum* [14]. The single copy fraction of *Physarum* DNA had not been analysed previously but had been predicted [11] to renature with a $c_0t_{1/2}$ = 500 mol · l^{-1} · s. We determined a value in that range.

The complexity of *Physarum* DNA was calculated from the unique DNA fraction to be about 130 times that of *E. coli* [4]. By comparison with a variety of other organisms [15] *Physarum* could be classified along with lower chordates. This myxomycete was clearly more complex than *Dictyostelium*, a cellular slime mould, which had a complexity of 11 times *E. coli* [16]. From the complexity of *Physarum* DNA, as estimated from several experiments, a genome

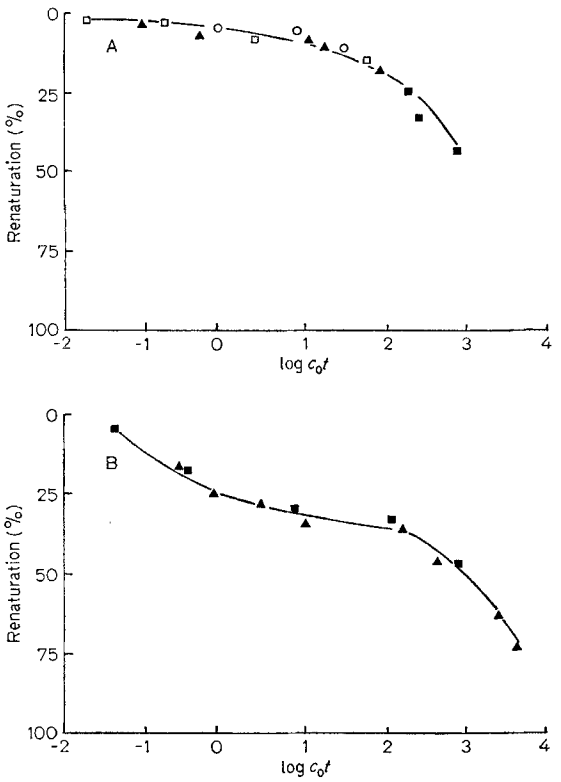

Fig. 2. *Renaturation of DNA labelled for fractions of S phase.* (A) DNA was labelled for the first quarter of S phase and analysed as in Fig. 1 B. (▲) 1000 μg/ml, (○) 1000 μg/ml (30% formamide), (□) 220 μg/ml, (■) 450 μg/ml. (B) DNA was labelled for the third (▲) and forth (■) quarter of S phase, 500 μg/ml

size or molecular weight of $2.5-7\times10^{11}$ could be calculated [1], a value very similar to the nuclear DNA content of 6×10^{11} mol · wt [6].

Furthermore, these results established conditions for high c_0t DNA hybridization experiments with *Physarum* [17].

Reassociation of DNA Labelled for Various Periods of S Phase

In *Physarum* replication starts immediately after mitosis and lasts for about 2.5 h [18]. We followed DNA synthesis by thimidine incorporation [18,19] for each consecutive quarter of S phase and determined renaturation kinetics by hydroxyapatite chromatography. In the second half of S phase (Fig. 2 B) we observed a pattern very similar to bulk DNA (Fig. 1) indicating a high proportion of repetitive DNA sequences (30%). Less than 10% repeated base sequences were detected in DNA labelled in the first quarter of S phase (Fig. 2 A). In the second quarter of S phase we observed 20% repetitive DNA. These results might indicate that unique DNA sequences were replicated throughout S phase while repeated DNA sequences were not synthesized in early S phase.

Evidence for sequential replication of nuclear DNA in *Physarum* had previously been obtained by density-shift experiments [20—22]. Also, early replicating DNA was shown to be denser [23] indicating d(G + C) contents of 45 or 40 mol/100 mol for early and late replicating DNA, respectively. Ribosomal DNA was repetitive [11] and a late-replicating fraction in *Physarum*. Part of this DNA was replicated even in G_2 phase [13,14,24,25]. In other organisms late replicating DNA had been associated with rDNA [26] or with heterochromatin [27,28] the possible site of some repetitive DNA [29—33]. These findings were in agreement with our reassociation data and suggested that sequential replication started mainly in euchromatic portions of the chromosomes. These may be the predominant sites of concomitant transcription early in the cell cycle of *Physarum* [34].

We thank Drs Evans (Cleveland) and Matthews (Portsmouth) for providing unpublished data and the *Deutsche Forschungsgemeinschaft* for financial support.

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