DIFFERENTIAL CLEAVATE OF *PHYSARUM* DNA FROM DISTINCT POINTS OF S PHASE BY RESTRICTION ENZYME Eco RI

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1. Introduction

Eucaryotic genomes contain unique and repeated base sequences which have been detected by an analysis of the renaturation kinetics of DNA [1].

If repetitive DNA segments contain recognition sites for restriction endonucleases, the cleavage products will appear in the digest in a higher proportion than that predicted from random cleavage and can be visualized as bands on polyacrylamide gels [2–4].

In this report we describe patterns of chromosomal DNA from *Physarum*, labeled for distinct periods of the naturally synchronous S phase, obtained with restriction enzyme Eco RI.

2. Materials and methods

2.1. *Culture and labeling procedures*

Synchronous macroplasmodia of *Physarum* were prepared and labeled with [^{3}H]thymidine (5–100) μCi/ml medium, 26 Ci/mmol, (Amersham) for specific periods of the S phase in the cell cycle as described previously [5–7]. Generation time was 10 h, S phase lasted 3 h. Cultures were labeled for complete S phase, or 0–15%, or 30–45%, or 50–100% of S phase.

2.2. *DNA preparation*

Nuclei were isolated according to Mohberg and Rusch [8] and main band DNA (1.700 g/cm^3) was prepared as we reported earlier [5,9]. DNA was purified on a preparative ethidium bromide/cesium chloride equilibrium gradient. Ethidium bromide was removed and the DNA was dialysed against 1/10 SSC, centrifuged on sucrose gradients and lyophilized. These procedures removed a contaminating nuclear polysaccharide and extrachromosomal nuclear satellite DNA molecules, as we have previously shown [9]. Specific activity of radioactive DNA preparations was $0.2–1 \times 10^5$ cpm/μg DNA.

2.3. *DNA degradation with restriction nucleases*

Physarum DNA preparations were cleaved for 48 h at 37°C with Eco RI in 200 μl of a buffer containing 10 mM Tris–HCl, pH 7.0, 10 mM $MgCl_2$. As controls, calf thymus DNA was treated with the restriction enzyme to check on the enzyme activity and to obtain an estimate of the molecular size of cleaved DNA segments from published results [2].

2.4. *Polyacrylamide gel electrophoresis*

Digested DNA samples were extracted once with phenol, lyophilized and dissolved in a borate buffer as described by Peacock and Dingman [10] containing 20% sucrose and 0.002% bromophenol blue. Samples of equal amounts of DNA (4 μg) were applied to 3% polyacrylamide gels (0.6×7 cm) and electrophoresis was performed for 2 h at 3 mA/gel at 14°C in borate buffer. Gels were stained with ethidium bromide (0.5 μg/ml) for 1 h, and photographed after u.v.-excitation of ethidium bromide fluorescence [11]. Distribution of radioactive DNA was analysed in slices of gels (3 mm wide) after hydrolysis of each slice in 200 μl of 10% TCA for one hour and adding a Triton based scintillator mixture, in a scintillation counter [12]. 70–90% of the radioactivity of undigested control DNA was recovered from the corresponding digested DNA

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samples after electrophoresis. Radioactivity in each slice was expressed as percentage of the total radioactivity eluted from each polyacrylamide gel.

3. Results and discussion

Untreated chromosomal DNA migrated as a single band as detected by ethidium bromide fluorescence (photographs not shown; fig.1, gel fraction 4). DNA treated with Eco RI revealed a broad band, as expected from random cleavage (gel fractions 4–7, with decreasing intensity of fluorescence) and one distinct band representing about 1% of the input DNA (gel fraction 15), with an approx. mol. wt. of 3.5×10^5.

Radioactive DNA, labeled for three generations or portions of one S phase was only detected as a single band (corresponding to the gel fraction 4, fig.1). After treatment with the restriction enzyme, we observed three fractions of low mol. wt. in the random labeled DNA (fig.1a), three similar fractions in the DNA labeled during 30–45% of S phase (fig.1c) and one band, which corresponds to the stained band, in the DNA labeled during 50–100% of S phase (fig.1d). DNA labeled during 0–15% of S phase revealed no distinct low molecular weight degradation products (fig.1b).

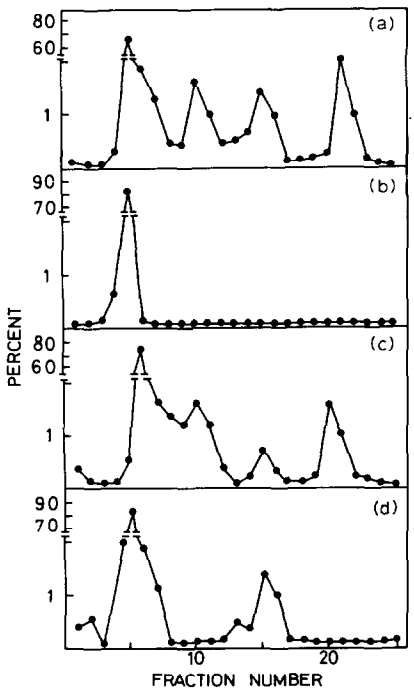

Fig.1. Degradation patterns of radioactive DNA from Physarum treated with Eco RI. (a) Fully labeled DNA, (b) DNA labeled from 0–15% of S phase, (c) DNA labeled from 30–45% of S Phase, (d) DNA labeled from 50–100% of S phase.

We interprete the results as an indication of repetitive DNA segments in the *Physarum* genome and further evidence that early replicating DNA contains less repetitive base sequences than later replicating DNA. We have previously detected differential renaturation kinetics for chromosomal DNA revealing only a small proportion of fast renaturating DNA segments in early S phase [5]. We have also observed that the nuclear RNA transcripts made in early S phase contain less repetitive base sequences that those made in G_2 phase [13]. Furthermore, we have obtained some evidence for a coupling of early replication during *Physarum* S phase with transcription [14]. We propose, as a working hypothesis, that a distinct portion of euchromatin is replicated and transcribed at the onset of S phase in *Physarum*.

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