DISCRETE LENGTH OF PALINDROMIC SEGMENTS FROM *TETRAHYMENA* DNA

S. N. BORCHSENIUS and N. A. MERKULOVA
Institute of Cytology, Academy of Sciences of USSR, Leningrad, 190121, USSR

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1. Introduction

The total macronuclear (Ma) DNA of *Tetrahymena* contains ≥4% of palindromic nucleotide sequences [1]. In ribosomal genes which are large (M_r 12.6 × 10^6), palindromes make up ~2% of the Ma DNA [2,3]. Recently a new extrachromosomal palindromic structure was found in *Tetrahymena* Ma DNA which is even larger (M_r 60 × 10^6) and constitutes ~1% of the total Ma DNA [4]. Here, comparatively small integrated palindromes from *Tetrahymena* DNA are described. The palindromic segments are discrete in length. Possible functions of these DNA segments are discussed in connection with some recombination events in the *Tetrahymena* genome [5].

2. Materials and methods

Axenic culture of *Tetrahymena pyriformis* (GL) was grown on a standard medium [6]. Macronuclei were isolated and DNA was extracted and purified as in [7].

DNA in solutions of 0.01 M NaCl was denatured at 100°C for 10 min then cooled rapidly in ice. Incubation with S1-nuclease from *Aspergillus oryzae* (Special Bureau of Biologically Active Substances, Novosibirsk) was done in 0.02 M Na-acetate buffer (pH 4.6) with 0.3 M NaCl and 0.01 M $ZnSO_4$. S1-nuclease (spec. act. 1.9 × 10^5 units/mg) was added (0.1–5 units/1 μg DNA) and the solution was incubated at 37°C for 45 min. Reaction was stopped by addition of EDTA. DNA was precipitated by 3 vol. ethanol and dissolved in the electrophoretic buffer.

To obtain the [^{14}C]DNA, [2-^{14}C]thymidine (52 μCi/mM, Isotop, USSR) was added to 1 μCi/ml. The level of [^{14}C]DNA hydrolysis with the S1-nuclease was calculated in % of the acid-insoluble radioactivity.

Electrophoresis of DNA was done in 5% polyacrylamide gel and 0.04 M Tris–acetate buffer (pH 7.8) with 0.005 M EDTA and 0.1% SDS [8]. DNA samples of 10–30 μg were applied and restriction fragments of phages DNA φX174/*Hae*III and λ/*Pst* were used as markers. Gels were stained with ethidium bromide and photographed under UV.

3. Results and discussion

Rapid cooling of a heated DNA solution gives a double-stranded (foldback) form of only those nucleotide sequences which were parts of palindromic structures before heating. The *Tetrahymena* Ma DNA treated in such a way was hydrolysed with S1-nuclease to remove single-stranded material. The enzyme concentration 3 units/μg DNA was chosen after some preliminary experiments with [^{14}C]DNA S1-nuclease hydrolysis. At this and higher enzyme concentrations 5% of the initial DNA remains in the insoluble state. It was shown that ≤1% of the native [^{14}C]DNA became acid soluble after S1-nuclease treatment.

Electrophoretic analysis of the foldback DNA revealed 3 areas (fig.1). Area I contains a major part of the material which is a foldback form of the ribosomal DNA and of another large extrachromosomal palindrome described in [4]. However, area I may contain some more foldback structures not identified yet because under the experimental conditions, the resolution of the gel is only good enough for fragments ≥3–2 kilobases long.

In area II there are 5 sharp bands. The positions of the bands are reproducible. These bands look as sharp as marker bands of the phage DNA restriction fragments and correspond to the 5 sets of double-stranded

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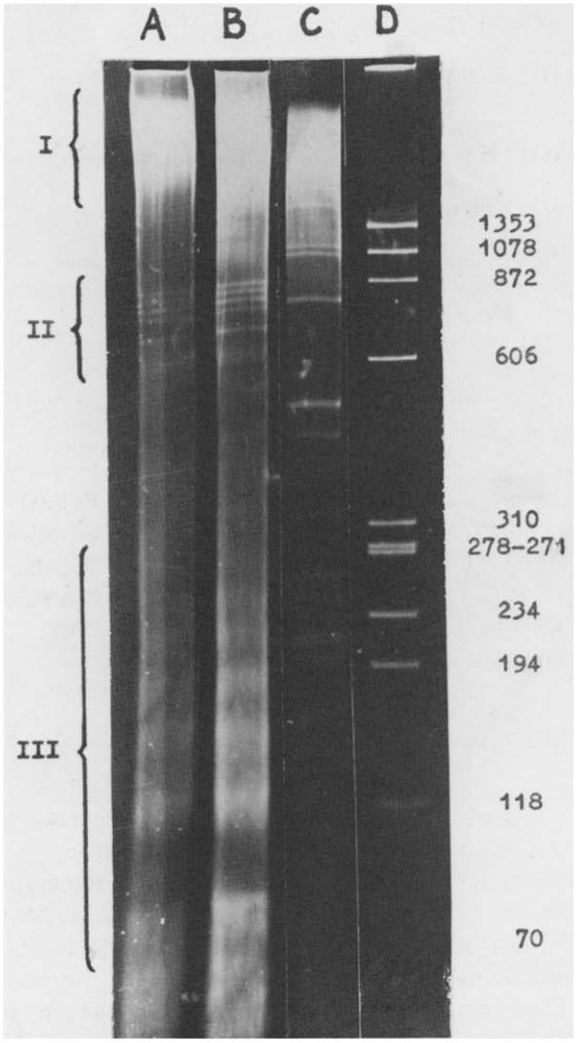

Fig.1. Electrophoresis of DNA fragments in 5% polyacrylamide gel. (A,B) Double-stranded fragments (hairpins) obtained as the result of *Tetrahymena* Ma DNA treatment with S1-nuclease after heating and cooling. (C) DNA restriction fragments of phage ϕX174/*Hae*III (D) DNA restriction fragments of phage λ/*Pst*. (I–III) Areas corresponding to foldback DNA of various lengths. Numerals denote the length of DNA fragments in basepairs.

DNA fragments, discrete in length, in the range 870–650 basepairs. Considering the sharpness of the bands and comparatively large sizes of the corresponding fragments it appears reasonable to assume that each of the 5 sets represent specific nucleotide sequences.

At the lower part of the gel (area III) 7–9 bands can be counted. These bands are not so sharp but are noticeable. They correspond to sets of double-stranded DNA fragments of 300–70 basepairs.

Isolation of extrachromosomal rDNA molecules was based on their comparatively high density in CsCl solutions relative to that of the bulk DNA [2,3]. The property of palindromic nucleotide sequences to fold back was used for detection and isolation of the second large extrachromosomal molecule from *Tetrahymena* [4]. The double-stranded molecules were then separated from single-stranded DNA by distribution in an aqueos two-phase system containing dextran and polyethylene glycol. Under such a separation double-stranded DNA hairpins of comparatively small size, which are parts of long nucleotide sequences get into the fraction of single-stranded molecules.

Digestion with the S1-nuclease permits us to collect all the hairpins formed both by extrachromosomal and integrated sequences not depending on their size and including hairpins with loops of any length.

We assume that the major part of the double-stranded fragments was formed by interaction of nucleotide sequences in the same DNA strand as a result of a first order reaction not dependent on concentration or time. Although the reassociation of some part of repetitive sequences is not completely excluded despite the absence of highly repetitive sequences in *Tetrahymena* DNA [1] and the digestion conditions (pH 4.6, 37°C) being far from optimal for annealing.

The fact that bands form during gel electrophoresis of the foldback DNA is an indication of the existence of palindromic segments of discrete length in the *Tetrahymena* genome. The sets of these palindromic segments seem to include identical or very similar DNA sequences. The integrated palindromes evidently are the parts of large DNA molecules $\geqslant 3-5 \times 10^7$ basepairs long which correspond to about the average length of *Tetrahymena* chromosomal DNA [9].

It is known that insertion sequences terminated in short palindromes in the DNA of some procaryotes are capable of translocating both inside the genome and from one genome to another [10,11]. A possible rearrangement of large palindromes in *Xenopus laevis* DNA was also suggested [12]. Intramacronuclear recombination events may explain the number of phenotypic combinations of several markers during vegetative propagation of *Tetrahymena thermophila* [5].

In case such recombination events also take place in the macronucleus of *T. pypiformis*, the sets of integrated palindromic segments we have found may serve as the molecular basis of the recombination process in the *Tetrahymena* genome. Furthermore, similarly to the central non-transcribed region of rDNA [13], short palindromes may serve as the unified origin sites for bidirectional replication of various genes.

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