BASE SEQUENCE DISTRIBUTION IN *TETRAHYMENA* MITOCHONDRIAL DNA

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1. Introduction

Electron microscopic studies of the mitochondrial DNA of *Tetrahymena pyriformis* have shown it to be a linear duplex of 15 μm length [1, 2]. Studies of the kinetics of reassociation of denatured mitochondrial DNA suggested a complexity of 30×10^6 daltons [3], in good agreement with the physical size. This DNA contains only 25% G + C and it seemed possible that extensive regions of d(A + T) sequences might be present. In yeast mitochondrial DNA, which is of even lower G + C content (17%) there is some evidence for the presence of such regions [4, 5]. This question is of some importance, since extended sequences of d(A + T) would be unlikely to be functional in specifying protein and would effectively reduce the genetic potential of the DNA. We have examined *Tetrahymena* mitochondrial DNA for the presence of d(A + T) clusters in two ways:

1) By thermal chromatography of sheared DNA on hydroxyapatite [6] and determination of the base compositions of the fragments and

2) by studying the binding of homopolyribonucleotides to the denatured DNA by the method of Hradecna and Szybalski [7]. We found no significant fraction of the DNA with less than 19% (G + C) but binding of poly U was observed: these two results suggest that although regions rich in dA:dT sequences are present, they are of relatively short extent.

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2. Materials and methods

2.1. *DNA preparation*

Tetrahymena pyriformis, strain T, was grown and mitochondrial DNA prepared as described previously [3]. Mitochondrial DNA, uniformly labelled with ^{32}P, was prepared from cells grown in 1% proteose peptone (Oxoid), supplemented with 10 μCi/ml carrier-free orthophosphate-^{32}P, and had an initial specific activity of 20,000 cpm/μg. DNA prepared from testes of *Cancer pagarus* [8] was used as a control.

2.2. *Thermal chromatography on hydroxyapatite*

DNA dissolved in 0.05 M sodium phosphate buffer, pH 6.8, was sheared by sonication to fragments 300–500 nucleotide pairs in length and was adsorbed to a column (1 × 2 cm) of hydroxyapatite, fitted with a water jacket. The temperature was raised to 60° and thereafter in 2° steps to 96°: at each temperature 2 aliquots of 2.5 ml of 0.1 M sodium phosphate, pH 6.8, were passed through the column. 10 min was allowed for conditions to stabilize at each temperature. Any DNA remaining bound at 96° was removed by washing with 0.4 M sodium phosphate, pH 6.8. The concentration of the fractions was measured either by E_{260} or, for the ^{32}P-DNA, by precipitating a portion onto a Millipore filter for liquid scintillation counting.

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2.3. *Base composition of fractions*

Fractions of the ^{32}P-DNA were hydrolysed to 5'-deoxynucleotides by the action of pancreatic deoxyribonuclease and venom phosphodiesterase [9]. The nucleotides were separated by ascending chromatography on Whatman No. 1 paper in propanol–4.5 M $(NH_4)_2SO_4$–0.1 M sodium phsophate, pH 6.8 (2:60:100 v/v/v). Spots were located by UV absorption, cut out and ^{32}P activity determined by liquid scintillation counting.

2.4. *Binding of polyribonucleotides*

The method of Hradecna and Szybalski [7] was followed. 2 μg of *Tetrahymena* mitochondrial DNA in 0.5 ml of 15 mM NaCl, 1.5 mM sodium citrate, pH 7.2 (0.1 SSC), was mixed with 5μg of polyriboadenylic acid (poly A), polyriboguanylic acid (poly G) or polyuridylic acid (poly U), heated to 100° for 5 min and cooled rapidly to 0°. 2 μg of native *Tetrahymena* mitochondrial DNA was added as a density standard (1.685 g cm^{-3}) and the density of the solution adjusted to 1.710 g cm^{-3} by adding solid CsCl. The solution was centrifuged to equilibrium at 45,000 rpm at 25°.

3. Results and discussion

The thermal chromatogram of *Tetrahymena* mitochondrial DNA is shown in fig. 1 and is rather asymmetric, a significant proportion (approx. 10%) being eluted at temperatures below 72°. In the comparable experiment with crab DNA approximately 25% was eluted in the range 62–72° and this fraction proved to be almost pure light satellite DNA (97% A + T). The base composition data for the mitochondrial DNA is also shown in fig. 1. No significant fraction contained less than 19% or more than 32% G + C and the composition is therefore rather homogeneous throughout the molecule, despite the broad thermal transition. In particular, those fractions which were eluted at the same temperature as crab light satellite DNA showed no evidence of an unusually low G + C content. The relationship between base composition and elution temperature shows a slope of 0.5% G + C/°C which can be compared with slopes of 1%/°C

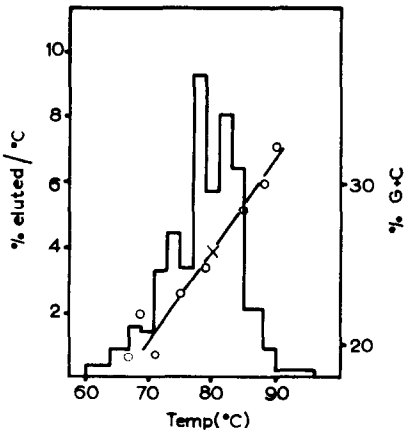

Fig. 1. Thermal chromatogram of *Tetrahymena* mitochondrial DNA and base compositions of the fractions. For details see sections 2.2 and 2.3. X indicates the composition of unfractionated mitochondrial DNA and is placed at the mean temperature of elution (T_m).

for bacteriophage T5 DNA (39% G + C) [6], 1.6%/°C for mouse DNA (44% G + C) [10] and 2%/°C for *E. coli* and bacteriophage λ DNAs (50% G + C) [6]. It is apparent that the relation between base composition and melting temperature is not a simple one. It seems to break down completely for DNA containing less than 20% G + C.

The fragment size of 300–500 nucleotide pairs corresponds to approximately 1% of the native length

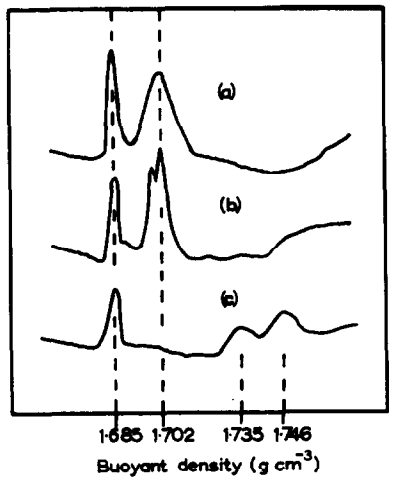

Fig. 2. CsCl gradient of *Tetrahymena* mitochondrial DNA. denatured in the presence of a) poly A b) poly G c) poly U. For details see section 2.4. The band at 1.685 g cm^{-3} is native mitochondrial DNA.

of the mitochondrial DNA and these experiments indicate that no stretches of this length are particularly rich in (A + T) sequences.

The results of the polynucleotide-binding experiments are shown in fig. 2. We expect that if binding of a polyribonucleotide to the DNA occurs, a large increase in buoyant density will be observed. With poly A and poly G no such increase is seen and all the DNA is at the density of denatured DNA. However, in the poly U experiment the DNA was displaced from the denatured density to give two bands at 1.735 g cm^{-3} and 1.746 g cm^{-3}. This indicates binding of poly U to dA clusters which are presumably unequally distributed on the two strands. The absence of binding of poly A is rather puzzling but was also observed by Borst and Ruttenberg [9] with mammalian mitochondrial DNA and may be related to the secondary structure of the polyribonucleotides. This experiment, then, suggests that regions of poly dA:dT do exist in the mitochondrial DNA. However, since no excess of A + T is observed in any of the thermal chromatogram fractions, these clusters of dA:dT must be quite restricted in length.

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