THE KINETIC COMPLEXITY OF EUGLENA GRACILIS CHLOROPLASTS DNA

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1. Introduction

The amount of double stranded DNA per Euglena gracilis chloroplast was estimated to be \(1 \times 10^{-14}\) g [1]. The question arises as to whether this analytical value has a correspondingly large kinetic complexity or whether Euglena chloroplast DNA, analogous to chloroplast DNA from some higher plants [2], contains a significant proportion of repeated nucleotide sequences.

To answer this question, we measured the reassociation rate of denatured chloroplast DNA using standardized conditions as given by Wettmur and Davidson [3]. From these results, we estimate Euglena chloroplast DNA to have a kinetic complexity equivalent to a nucleotide sequence of \(1.8 \times 10^8\) daltons, which is approximately one-thirtieth of the analytical complexity.

2. Materials and methods

Euglena gracilis Klebs (z-strain) cells were grown routinely under autotrophic conditions, harvested, washed and stored at \(-60^\circ\) as reported earlier [4]. Chloroplast DNA and nuclear DNA were isolated and purified as described in detail [5]. The purity of chloroplast and nuclear DNA was tested by measuring the buoyant density in neutral CsCl (Spinco Model E, An-D rotor, 12 cm centriplate) and was considered satisfactory when, at a total load of 5 \(\mu\)g per ml, only one band was detectable either at \(\rho = 1.685\) g/ml for chloroplast DNA or \(\rho = 1.708\) g/ml for nuclear DNA [1, 6, 7]. The molecular weights of the various DNA components were calculated according to Studier [8] from S values determined by band-centrifugation [9]. The rate of DNA reassociation was monitored optically at 260 nm using a Gilford Model 2000 with temperature controlled cuvette chamber and automatic recording device.

E. coli DNA was isolated according to Marmur [10]. Bacteriophage T4 DNA was a gift from Dr. Hazekorn, University of Chicago.

3. Results and discussion

It was already known from buoyant density measurements (CsCl) that Euglena chloroplast DNA, contrary to nuclear DNA, renatures rapidly and rather extensively [5]. The kinetics of the reassociation process are shown in fig. 1 where the relative change in hyperchromicity is plotted versus time. Under these experimental conditions Euglena chloroplast DNA and bacteriophage T4 reassociate at an almost identical rate. Increase in Na\(^+\) concentration from 0.15 to 1.0 M increases the reassociation rate as expected [3].

Euglena nuclear DNA renatures to a small extent and very rapidly at the beginning of the process but the reaction becomes very slow afterwards.

In fig. 2, a second order rate plot [3] of the reassociation data is shown. Euglena nuclear DNA is omitted but E. coli DNA is added for comparison. Over the time shown, Euglena chloroplast DNA yields a straight line under both ionic conditions indicating a rather homogeneous type DNA. This is contrary to results obtained with lettuce chloroplast DNA [2] where a fast and a slow component were found. However, for chloroplast DNA isolated from tobacco
plants only one type of DNA was found which  
renatured at a rate equivalent to a nucleotide sequence  
complexity of approximately $2 \times 10^8$ daltons [11].

In table 1 the pertinent data are summarized. The  
kinetic complexity is calculated according to Wetmur  
and Davidson [3] using the $k_2$ from the reassociation  
experiments at 1 M Na$^+$. According to this, *Euglena*  
chloroplast DNA ($G + C = 26\%$, $\rho = 1.685$ g/ml,  
neutral CsCl) has an average kinetic complexity of  
$1.8 \times 10^8$ daltons. This value is about 30 times  
smaller than the reported analytical value [1]. We  
believe that a gross error in our renaturing  
experiment can be excluded especially since our results for  
both bacteriophage T4 and *E. coli* DNA match well
the published values [3]. We may, therefore, conclude that either the reported analytical value is too high by at least one order of magnitude or the chloroplast DNA has a high frequency of repetitious nucleotide sequences (e.g., polyploid).

The question as to the length of the chloroplast DNA molecule(s) remains open. It was concluded from UV-irradiation studies [12] that mature chloroplasts of *Euglena* contain three DNA entities. Provided these three “chromosomes” are equal in size, an average molecular weight of $2 \times 10^9$ results. Ray and Hanawalt [6] using sucrose gradients calculated that *Euglena* chloroplast DNA had a molecular weight of $2 \times 10^7$ (largest component). We measured the S value by band-centrifugation in 1 M NaCl and calculated a molecular weight of $1.2 \times 10^7$. However, we have good reasons to believe that our chloroplast DNA preparations are fragmented [13] and some of our DNA/RNA hybridization results can best be explained by assuming that intact *Euglena* chloroplast DNA has a molecular weight in the range of $10^8$ daltons. Finally, it is noteworthy that *Euglena* chloroplast DNA contains between 20 to 30 cistron copies coding for 23 S/16 S chloroplast rRNA [5, 14]. This figure matches, maybe accidentally, the number of nucleotide sequence repetitions.
Table 1

Complexity in molecular weight units calculated from reassociation rates [3].

<table>
<thead>
<tr>
<th>DNA</th>
<th>pH 13</th>
<th>$k_2$ (mol$^{-1}$·sec$^{-1}$)</th>
<th>$\frac{5.5 \times 10^8 (S_{20,w}^{1.25})}{k_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euglena chloroplast</td>
<td>16.8</td>
<td>101</td>
<td>1.8 $\times$ 10$^8$</td>
</tr>
<tr>
<td>Bacteriophage T4</td>
<td>15.1</td>
<td>120</td>
<td>1.4 $\times$ 10$^8$</td>
</tr>
<tr>
<td>E. coli</td>
<td>14.5</td>
<td>5</td>
<td>3.0 $\times$ 10$^9$</td>
</tr>
</tbody>
</table>

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References