Kinetic Complexity of *Tetrahymena pyriformis* Nuclear Deoxyribonucleic Acid

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Tetrahymena pyriformis in common with other ciliates has two types of nuclei, a micronucleus and a macronucleus. The micronucleus is active only during the sexual cycle of the organism, and cells that have lost the micronucleus survive through many years of vegetative growth without apparent loss of function. The macronucleus is the major site of RNA synthesis in the cell and the information contained in this nucleus seems to be sufficient for the maintenance of vegetative growth. Genetic experiments show that the micronucleus is diploid and its replication follows the normal mitotic and meiotic patterns. Replication of the macronucleus is usually considered to be amitotic and chromosomes have not been detected. The amount of DNA in the macronucleus is not constant and may differ by up to 20% between sister cells (Cleffman, 1968). Woodward, Gorovsky & Kaneshiro (1968) find that the macronucleus contains about 20 times the micronuclear complement, whereas Cleffman (1968) finds the average value to be 40 times. The organization of the DNA in the macronucleus has been thought to be either a completely random population of DNA molecules replicating independently (the 'grab bag' hypothesis; Kimball, 1943) or a collection of replicating diploid entities or subnuclei (Sonneborn, 1947).

The subnuclear hypothesis predicts a ploidy level of 90, i.e. 45 diploid sets per macronucleus (Nanney, 1964), and since the directly determined ratios are in conflict we have approached the determination of the DNA content of the macronucleus in a different manner by measuring the kinetic complexity of the DNA and the total DNA content of the cell.

Materials and methods. *T. pyriformis* strain T (an amiconucleate strain from the Culture Collection of Alga eand Protozoa, Botany School, Cambridge) and syngen 1 inbred family A (a gift from Dr D. L. Nanney) were grown and harvested as described by Flavell & Jones (1970). Nuclei were prepared by a modification of the method of Mita, Shiomi & Iwai (1966). A 10% cell suspension in 0.25 M-sucrose-1 mM-MgCl₂-3 mM-CaCl₂-10 mM-tris-HCl buffer, pH 7.6, was lysed by the addition of Nonidet P 40 (a non-ionic detergent) to a final concentration of 0.5% (v/v). The crude pellet of

nuclei obtained by centrifuging at 2000g for 10 min was taken up in the same buffer, made 60% (w/w) with respect to sucrose and centrifuged at 45 000g for 60 min in a Sorvall RC-2B refrigerated centrifuge. The nuclear pellet was resuspended in the 0.25 M-sucrose buffer and centrifuged at 1000g for 10 min. The nuclei in the final pellet were almost completely free from cytoplasmic contamination and, in particular, no mitochondria were present. DNA was prepared by the method of Suyama (1966) and freed from oligoribonucleotides by passage through a column of Sephadex G-100 in 30 mM-sodium phosphate buffer, pH 6.8. A further purification was achieved by adsorbing the DNA on a column of hydroxyapatite, washing with 75 mM-sodium phosphate buffer, pH 6.8, until the E_{260} value fell below 0.05 and then eluting the DNA with 0.4 M-sodium phosphate.

The kinetics of renaturation were studied as follows. DNA was sheared to various molecular weights in a Branson Soniprobe 1130/1A (Dawe Instruments Ltd., London W.3, U.K.). The DNA was denatured by either heat or alkali and transferred to a quartz cuvette maintained at $(T_m - 25)^\circ\text{C}$, where T_m is the 'melting' temperature. The decrease in E_{260} with time was followed in the Unicam SP.800 spectrophotometer with a scale-expansion device with an external recorder. From the continuous trace a second-order rate constant for the renaturation was calculated by the method of Wetmur & Davidson (1968). Total DNA of the cell was determined by the diphenylamine method (Ogur & Rosen, 1950) with bacteriophage T4 DNA as a standard. Cells were counted in a haemocytometer.

Sedimentation coefficients of the various DNA samples were determined in 0.9 M-NaCl-0.1 M-NaOH at a concentration of 60 µg/ml at 50 000 rev./min in an MSE analytical ultracentrifuge.

Hydroxyapatite (Bio-Gel HTP) was obtained from BioRad Laboratories, Richmond, Calif., U.S.A.). All other chemicals were of reagent grade.

Results and Discussion. The second-order rate constants (k_2) for the renaturation of *T. pyriformis* strain A and strain T DNA are given in Table 1. Rate constants were also determined for bacteriophage T4 DNA and *Escherichia coli* DNA. The

Table 1. *Renaturation constants and complexity of T. pyriformis DNA*

Renaturation kinetics were followed at ($T_m - 25$)°C in 1M-NaCl-5mM-tris-HCl buffer, pH 7.5. Complexity was calculated relative to bacteriophage T4 DNA (complexity 1.3×10^8 daltons).

Source of DNA	k_2 (l mol ⁻¹ s ⁻¹)	$s_{20,w}^{PH13}$ (S)	$k_2/s^{1.25}$	$10^{-10} \times$ Complexity (daltons)
<i>T. pyriformis</i> strain A	0.063, 0.062	4.5	0.0084	4.3
<i>T. pyriformis</i> strain T	0.057	4.0	0.0100	3.7
	0.140	9.6	0.0084	4.3
Bacteriophage T4	21.0	5.0	2.81	(0.013)
<i>E. coli</i>	1.50	5.0	0.200	0.18

constant calculated for the nuclear DNA sample is that of the final slope of the second-order renaturation rate plot. An initial rapid renaturation is observed (approx. 5%, as calculated from extrapolating the final slope to zero time) and this we attribute to reiterated DNA. Calculations of the degree of reiteration show that this component does not contribute significantly to the total genome size in the nucleus, and we have therefore ignored this in the calculation. The renaturation of the second, and major, component has been followed both by optical hypochromicity (eight experiments) and by measurements of the fraction of renatured DNA at various time-intervals by hydroxyapatite column chromatography (R. A. Flavell & I. G. Jones, unpublished work) and no deviations from linearity have been observed for up to 70% of the total reaction over long time-intervals.

Wetmur & Davidson (1968) have shown that:

$$k_2 = \frac{5.5 \times 10^8 \cdot (s_{20,w}^{PH13})^{1.25}}{N_D}$$

where N_D is the complexity in daltons and s is the alkaline sedimentation coefficient. However, the value of the numerical constant varies in a manner related to the guanine and cytosine content of the DNA. For bacteriophage T4 DNA (guanine + cytosine content 34%) the kinetic complexity is too high by approx. 40% (Wetmur & Davidson, 1968), but Borst (1969) has shown that for guanine + cytosine contents below this value the deviations change less rapidly. We have therefore calculated the kinetic complexity of *T. pyriformis* DNA (guanine + cytosine content 25%) relative to bacteriophage T4 DNA. The error introduced by the difference in guanine + cytosine content is probably not more than 10%. The ratio $k_2/s^{1.25}$ was calculated for each sample and from this the complexity relative to bacteriophage T4 DNA, assuming that this has a complexity of 1.3×10^8 daltons (Table 1). The value is approx. 4×10^{10} daltons and no significant difference is observed between strain A and strain T. The diploid micronucleus must therefore contain about 8×10^{10} daltons of DNA.

The total DNA of cells in exponential-phase

growth was found to be 6.7×10^{12} daltons, and this agrees well with the results of Scherbaum (1957). About 10% of this DNA is cytoplasmic and hence the content of the exponential-phase macronucleus is estimated to be 6.0×10^{12} daltons. Studies on synchronized cultures of *Tetrahymena* by Zeuthen and others (Zeuthen, 1964) have shown that exponential-phase cells contain about 1.4 times as much DNA as cells in which a new round of replication is about the start (i.e. the G-1 phase). We therefore estimate the DNA content of the G-1 macronucleus as 4.3×10^{12} daltons. This suggests that the G-1 macronucleus contains about 100 copies of the genome or 50 diploid sets. This agrees well with the microspectrophotometric measurements made by Cleffman (1968), but is twofold greater than the estimate made by Woodward *et al.* (1968).

When the phenotype of a heterozygote clone of *T. pyriformis* is examined throughout the vegetative growth of the clone, cells that are phenotypically indistinguishable from one or other of the parents appear at a constant rate.

Allen & Nanney (1958) have interpreted their data for the segregation of mating types by means of a computer analysis (Schensted, 1958) based on a macronucleus composed of differentiated diploid subnuclei. The best fit for the data suggested 45 subnuclei in the G-1 macronucleus. Subsequent work with other alleles gave identical results. Our results give no direct evidence for the existence of subnuclei as such, but do show that the hypothesis is compatible with the DNA contents.

The value of 4×10^{10} daltons for the complexity of *T. pyriformis* nuclear DNA lies between the values for *Saccharomyces carlsbergensis* and *Pisum sativum* (Retèl & Planta, 1968), which to the authors seems reasonable on the grounds of evolutionary status. The value obtained by Woodward *et al.* (1968) is 1.25×10^{11} daltons, of the same order as that of *Drosophila melanogaster* (Ritossa & Spiegelman, 1965), which seems inherently unlikely.

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