Biochem. J. (1970) **116**, 811–817
Printed in Great Britain

Mitochondrial Deoxyribonucleic Acid from *Tetrahymena pyriformis* and its Kinetic Complexity

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(*Received* 24 *November* 1969)

1. Mitochondrial DNA from *Tetrahymena pyriformis* strain T has a buoyant density (ρ) of 1.685 compared with ρ 1.688 for whole cell DNA. Mitochondrial preparations from *T. pyriformis* strain W show an enrichment of a light satellite (ρ 1.686), although this is not obtained free from nuclear DNA (ρ 1.692). 2. *T. pyriformis* mitochondrial DNA renatures rapidly and the kinetics of this process indicate a complexity of approx. 3×10^7 daltons. 3. The base-pairing in the renaturation product is of a precise nature, since the 'melting' temperature (80.5°C) is indistinguishable from that of the native DNA (80.5°C). 4. Centrifugation of mitochondrial DNA in an alkaline caesium chloride density gradient gives two bands, implying the separation of the complementary strands.

The presence of DNA in mitochondria of a wide variety of cell types has been confirmed by many workers (Nass, Nass & Afzelius, 1965), and in a number of cases the mitochondrial DNA has been extracted and its properties have been examined. The relationship of the mitochondrial DNA to the nuclear DNA is of particular interest and certain generalizations can be stated: (*a*) mitochondrial DNA may differ from nuclear DNA in buoyant density; (*b*) the content of genetic information in the mitochondrial DNA is lower than in the nuclear DNA by several orders of magnitude; this can be inferred from the observation that denatured mitochondrial DNA will renature readily whereas nuclear DNA renatures very slowly (Dawid & Wolstenholme, 1968); (*c*) mitochondrial DNA is not complementary to nuclear DNA (Dawid & Wolstenholme, 1968) and hence contains genetic information not represented in the nuclear DNA.

The nature of mitochondrial DNA has been investigated by using electron microscopy. The mitochondrial DNA of mammals and birds (Kroon, Borst, van Bruggen & Ruttenberg, 1966), amphibians (Dawid & Wolstenholme, 1967) and some invertebrates, e.g. sea urchin (Piko, Tyler & Vinograd, 1967), has been found in the form of a closed circular duplex approx. 5μm in length, corresponding to a molecular weight of 10^7 daltons. However, the estimated number of DNA molecules per mitochondrion varies from one or two (Piko *et al.* 1967) to 14 (Clayton & Vinograd, 1967). Mitochondrial DNA of *Neurospora crassa* (Luck & Reich, 1964) and yeast (Sinclair, Stevens, Sanghavi & Rabinowitz, 1967) is of a similar size but apparently exists as linear filaments, although Avers (1967) has consistently shown circular DNA in yeast mitochondria. In the red bean *Phaseolus vulgaris* (Wolstenholme & Gross, 1968), and the ciliated protozoon *Tetrahymena pyriformis* (Suyama & Miura, 1968) electron micrographs of mitochondrial DNA revealed linear filaments of 19.5μm and 17.6μm mean length respectively. These molecules are thus three or four times the size of the animal mitochondrial DNA species and do not seem to be circular.

Suyama (1966) isolated mitochondrial DNA from *T. pyriformis* with a relatively uniform sedimentation coefficient of approx. 40S, corresponding to 4.0×10^7 daltons, and Suyama & Preer (1965) showed that a single mitochondrion of *T. pyriformis* contained 2.4×10^8 daltons of DNA. Each mitochondrion must therefore contain some six or seven DNA molecules, but whether the genetic unit or genome corresponds to this small molecule (3.5×10^7–4.0×10^7), to some fraction of it, to the total DNA of the mitochondrion (2.4×10^8) or even to some higher multiple of this has not been determined. This length, which we can call the genetic complexity, cannot be determined directly, but it has been demonstrated (Britten & Kohne, 1966; Wetmur & Davidson, 1968) that the rate of renaturation of denatured DNA under controlled conditions is a sensitive measure of the complexity of the DNA. This kinetic complexity is essentially identical with the genetic complexity for the DNA of procaryotic organisms, whereas in the nuclear DNA of eucaryotic organisms several components of differing complexity can be distinguished. This has led to the idea that the eucaryotic genome contains certain repetitive segments. The DNA of

chick liver mitochondria has been studied by this technique (Borst, Ruttenberg & Kroon, 1967) and a unique kinetic complexity of 8×10^6 daltons has been calculated. This value corresponds to the length of the circular DNA molecules measured in electron micrographs. We have studied the kinetic complexity of *T. pyriformis* mitochondrial DNA by this method.

MATERIALS AND METHODS

Maintenance and growth of the organism. Amicronucleate strains of *T. pyriformis*, T, W, L-I, L-II, GP, G1-R and GL, were obtained from the Culture Collection of Algae and Protozoa, Botany School, Cambridge, U.K. A single micronucleate strain of syngen 1 (inbred family A) was obtained from Dr D. L. Nanney. Cultures were maintained axenically at room temperature (22°C) in a medium containing 10g of proteose peptone (Oxoid Ltd., London E.C.4, U.K.) and 2g of yeast extract (Difco Laboratories, Detroit, Mich., U.S.A.)/l and were subcultured weekly. Large-scale cultures were grown in a medium containing (g/l): proteose peptone (Oxoid), 20; yeast extract (Difco) 2; Na_2HPO_4, 1; KH_2PO_4, 1; NaCl, 2; $MgSO_4,7H_2O$, 0.05. A portion of an overnight culture was added to 2l of this medium in a 4l wide-necked conical flask and incubated at 28–30°C on a Gyrotory shaker (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.) for 24h. A yield of 5–15ml of packed cells/l was obtained, depending on the strain used.

Preparation of mitochondria. The method used is essentially that of Suyama & Preer (1965). Cells from 4l or more of medium were harvested in a Griffin–Christ continuous-flow centrifuge at 3000*g* with a flow rate of 400ml/min. The cell suspension was concentrated in an MSE oil-testing centrifuge at 750*g* for 15min. Smaller volumes of cells were harvested in an MSE Mistral 6L centrifuge at 800*g* for 10min. The cells were suspended in 10 vol. of 0.3M-sucrose–2mM-EDTA–10mM-tris–HCl buffer, pH7.2, and homogenized with an Ultra-Turrax tissue disintegrator (type TP18/2; Janke und Kunkel K. G., Staufen i. Br., Germany) until almost complete lysis had occurred. The homogenate was centrifuged at 500*g* for 7½min and the pellet (containing whole cells and nuclei) was discarded, and this step was repeated. The supernatant extract was centrifuged at 5500*g* for 7½min to sediment the mitochondria. The pellet was resuspended in the same volume of the homogenization medium and again centrifuged at 5500*g* for 7½min. This step was repeated twice. The final pellet consisted of mitochondria (staining with Janus Green B) with very slight contamination by oral plates and small pellicle fragments. Nuclei were not found in the final mitochondrial pellet.

Isolation of DNA. Mitochondrial DNA and whole-cell DNA were extracted by the method of Suyama (1966) except that phenol was removed by ether extraction. DNA samples were concentrated when necessary by rotary evaporation at 30°C under vacuum. DNA was stored in SSC*. Further purification of DNA was obtained by filtration through a column (2cm × 25cm) of Sephadex G-75 (equilibrated with SSC) followed by adsorption on a column (1cm × 3cm) of MAK. Alternatively the DNA was purified on a preparative CsCl gradient. *Micrococcus lysodeikticus* DNA and *Escherichia coli* DNA were prepared by the phenol method of Thomas, Berns & Kelly (1966). Bacteriophage T4 was purified by differential centrifugation and bacteriophage T4 DNA was prepared by phenol extraction.

MAK column chromatography. Methylated albumin was prepared by the method of Mandel & Hershey (1960) and the stepwise elution procedure of Sueoka & Cheng (1962) was followed. The NaCl buffers were all prepared in 0.05M-sodium phosphate buffer, pH6.7. After the DNA was applied to the column, 0.4M-NaCl was added and the column washed until the E_{260} decreased to less than 0.10. The DNA was then eluted with 0.8M-NaCl.

Preparative caesium chloride-density-gradient centrifugation. DNA solutions were adjusted to a density of 1.686–1.688 with CsCl (AnalaR) and centrifuged for 48–60h at 42000rev./min in a 10 × 10ml fixed-angle rotor (type 2410) in an MSE Superspeed 50 centrifuge (Flamm, Bond & Burr, 1966). Each tube contained 60μg of DNA in 4.5ml and was filled completely with liquid paraffin. Fractions were collected by piercing the tube.

Analytical caesium chloride-density-gradient centrifugation. The methods of Meselson, Stahl & Vinograd (1957) and Schildkraut, Marmur & Doty (1962) were followed. An MSE analytical ultracentrifuge was used at approx. 45000rev./min. Each cell was equipped with a 1° negative upper window and a 10mm centre-piece. *M. lysodeikticus* DNA (density 1.731, calibrated against *E. coli* DNA, density 1.710) was used as a density standard in neutral gradients. Alkaline CsCl gradients were prepared by adding 0.2ml of DNA solution to 0.8ml of a solution of CsCl made alkaline (pH12) with NaOH. The density of the final solution was 1.750. Centrifugation was continued for 18–24h at 25°C. Photographs were taken with u.v. light (253nm) and the negatives were scanned with a Joyce–Loebl double-beam microdensitometer (Joyce, Loebl and Co., Gateshead, Co. Durham, U.K.). Buoyant densities were calculated from the formula of Sueoka (1961). Alkaline buoyant densities were calculated from the initial density of the solution. No corrections for pressure effects were made.

Sedimentation velocity. Alkaline sedimentation coefficients of sheared DNA were measured by boundary sedimentation at concentrations of 10–80μg/ml at 50000rev./min in 0.9M-NaCl–0.1M-NaOH.

Thermal denaturation of DNA. The 'melting' point, T_m (defined as that temperature at which half the total increase in E_{260} has occurred), was determined in SSC. A Unicam SP.800 recording spectrophotometer was used with electrical heating (SP.874) and the high-temperature cell housing. The scale-expansion device was used in conjunction with a Servoscribe (Kelvin Electronics Co.) linear external recorder. The temperature of the sample was measured by a thermocouple in the sample cell connected to a Pye Scalamp galvanometer.

Thermal renaturation of DNA. DNA for renaturation studies was denatured by heating at 100°C for 5min in the spectrophotometer cuvette. The temperature of the cuvette was rapidly decreased by circulating cold water through the cell housing. When the temperature

* Abbreviations: SSC, standard saline citrate (0.15M-NaCl–15mM-sodium citrate, pH7.2); MAK, methylated albumin on kieselguhr.

(T_m−25°C) at which the renaturation was to be followed was approached the circulating water was expelled with air and electrical heating continued. Measurement of renaturation rates was made in either 2×SSC or 1M-NaCl–0.2mM-EDTA–50mM-tris–HCl buffer, pH7.8. Second-order renaturation constants were calculated as described by Wetmur & Davidson (1968).

Degradation of DNA. DNA was sheared by sonication with a Branson Soniprobe (100W type 1130/1A; Dawe Instruments Ltd., London W.3, U.K.).

Measurement of total carbohydrate. The anthrone method as modified by Binnie, Dawes & Holms (1961) was used, with D-glucose as a standard.

Special chemicals. Deoxyribonuclease (20–30% powder), ribonuclease and CsCl solution (60%, w/w) were obtained from BDH (Chemicals) Ltd., Poole, Dorset, U.K. Ribonuclease T_1 from *Aspergillus oryzae* and bovine serum albumin (Fraction V) were obtained from Sigma (London) Chemical Co., London S.W.6, U.K. Glucoamylase was a gift from Dr W. Banks. All other chemicals were of analytical grade.

RESULTS

Caesium chloride-density-gradient analysis. To find strains possessing satellite DNA bands, samples of whole-cell DNA of several strains of *T. pyriformis* were examined by caesium chloride-density-gradient centrifugation (Table 1). Strains L-I, T, W and GL showed a satellite of lower buoyant density than the main component. A satellite of higher buoyant density than the main band was found in strains L-II, GL, T and W. Whole-cell DNA preparations from several strains were contaminated with a band of lower buoyant density (approx. 1.66) than the DNA bands. This gave a large refractive-index difference when viewed with schlieren optics and was totally removed by treatment with glucoamylase, a mould enzyme capable of hydrolysing α-(1→4)-glucosyl linkages. Further, the carbohydrate content of the DNA sample diminished on treatment with glucoamylase, as measured by the anthrone method. Thus this band can be assumed to be glycogen. A similar band has been reported by Brunk & Hanawalt (1966).

Table 1. *Buoyant-density values for whole-cell DNA from* T. pyriformis

DNA was prepared by phenol extraction and centrifuged to equilibrium (20h) in a CsCl density gradient at 45000rev./min at 25°C in an MSE analytical ultracentrifuge. The u.v. photographs were scanned by a Joyce–Loebl mark III CS microdensitometer and buoyant densities calculated by using the equation of Sueoka (1961).

Strain	Light satellite	Main band	Heavy satellite
Syngen 1; family A	—	1.685	—
GL	1.684	1.686	1.693
Gl-R	—	1.689	—
GP	—	1.688	—
L-I	1.684	1.687	—
L-II	—	1.688	1.696
T	1.685	1.688	1.697
W	1.686	1.692	1.700

Mitochondrial preparations were made from strains, T, W and A. With the last two strains difficulty was found in isolating mitochondria owing to the great viscosity of the extracts. Suyama (1966) has also observed this with several strains. However, mitochondria could be prepared relatively easily from strain T and this strain was used in the remainder of our investigation. The mitochondrial fraction of strain W gave an enrichment of the light satellite (ρ 1.686), whereas the same fraction of strain A gave a single band at the same density as the whole-cell DNA (ρ 1.685).

DNA from mitochondria of strain T showed a single sharp band in the caesium chloride density gradient at ρ 1.685 (Fig. 1*b*). This compared with the whole-cell major band at ρ 1.688 (Fig. 1*a*). Incubation of the mitochondria with deoxyribonuclease (50μg/ml in 5mM-magnesium chloride for 30min at 37°C) did not affect the band, showing that the mitochondria as isolated in our procedure are impermeable to deoxyribonuclease. When denatured by heat (100°C for 5min) the mitochondrial DNA band increased in buoyant density to 1.701 (Fig. 1*c*). When whole-cell DNA was subjected to the same conditions its buoyant density was 1.708. When the mitochondrial DNA was incubated at 60°C for 2h in 2×SSC its buoyant density returned to a value almost the same as that of native mitochondrial DNA (ρ 1.686) (Fig. 1*d*). The band-width of the renatured material was extremely small. The buoyant density of denatured whole-cell DNA did not change under these conditions, although when the annealing period was increased to 18h renaturation was observed (ρ 1.695). When a mixture of mitochondrial DNA and whole-cell DNA was annealed for 18h both bands renatured separately to values of 1.686 and 1.695 respectively. Apparently no common duplexes are formed. Centrifugation of mitochondrial DNA to equilibrium in an alkaline caesium chloride density gradient gives two bands at ρ 1.750 and 1.756.

Thermal denaturation. It was found that DNA samples prepared as described in the Materials and Methods section gave low hyperchromicities when heated above the 'melting' point (T_m). Since this was thought to be due to low-molecular-weight RNA fragments resistant to pancreatic ribonuclease, a treatment with T_1 ribonuclease was included in several preparations. Addition of T_1 ribonuclease subsequent to pancreatic ribonuclease produced a further increase in E_{260} indicating that the preparation contained oligoribonucleotides

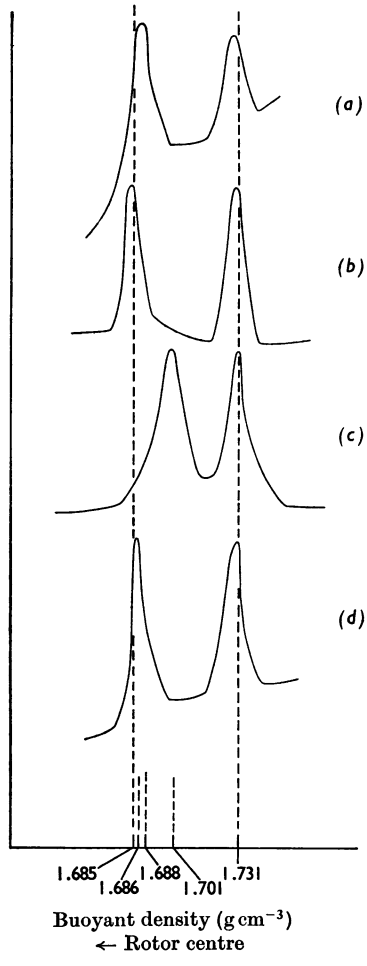

Fig. 1. Microddensitometer tracing of u.v. photographs of isopycnic CsCl-density-gradient centrifugation of DNA from strain T. *Micrococcus lysodeikticus* DNA is at 1.731 g cm^{-3}. All samples had an initial density of 1.710 g cm^{-3} and were centrifuged at 45000 rev./min. for 20–25 h at 25°C. (*a*) Whole-cell DNA (ρ1.688); (*b*) mitochondrial DNA (ρ1.685); (*c*) denatured mitochondrial DNA (ρ1.701); (*d*) denatured mitochondrial DNA, renatured for 130 min in 2 × SSC at 60°C (ρ1.686).

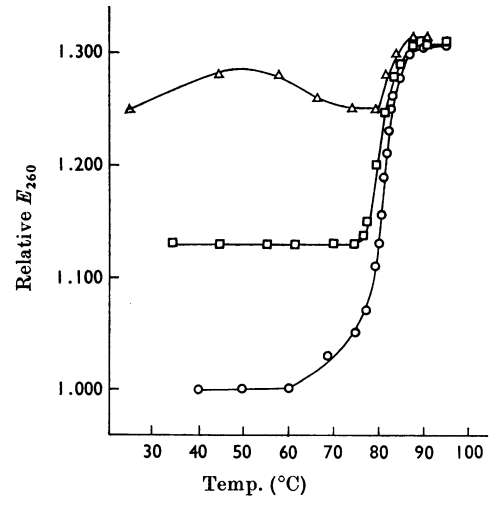

Fig. 2. Thermal denaturation profiles. DNA solutions (about 20 μg/ml) in SSC were heated at each temperature for 10 min. The temperature was measured with a calibrated thermocouple in the sample cell. (*a*) ○, Native mitochondrial DNA; (*b*) □, renatured mitochondrial DNA; (*c*) △, mitochondrial DNA heated at 100°C for 10 min, then quickly cooled.

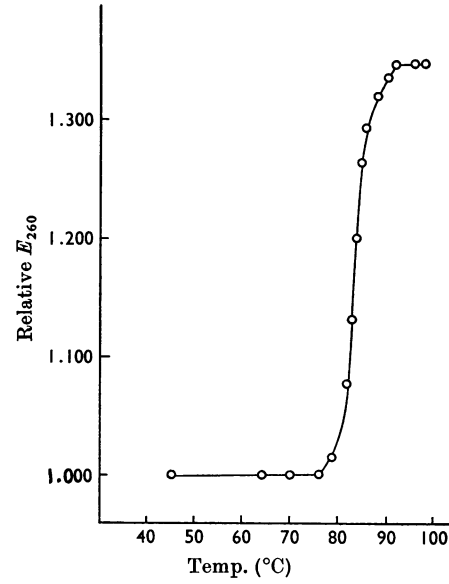

Fig. 3. Thermal denaturation profile of whole-cell DNA of *T. pyriformis* strain T. Conditions are as for Fig. 2.

(presumably guanine-rich) capable of being hydrolysed by this enzyme; this treatment still did not render the contaminants removable by dialysis. Application of DNA samples to a Sephadex G-75 column gave the DNA in the excluded volume and a later peak (up to 50% of total) that also had the absorption spectrum of a nucleic acid. The DNA solution from the excluded volume had a hyperchromicity consistent with that of a double-stranded DNA (33–40%). Further purification by MAK-column chromatography or preparative caesium chloride-density-gradient centrifugation was carried out as a routine.

T. pyriformis strain T mitochondrial DNA had T_m 80.5°C in 1 × SSC and a hyperchromicity of 32% (Fig. 2*a*). Whole-cell DNA from the same strain had T_m 83°C and a hyperchromicity of 35% (Fig. 3). The thermal-denaturation profiles of renatured and previously denatured DNA (Figs. 2*b* and 2*c* respectively) were measured to ensure that the optical effects observed on renaturation could be explained by base-pairing phenomena. Renatured *T. pyriformis* mitochondrial DNA had T_m 80.5°C, identical with that of native DNA. Denatured DNA showed little hyperchromicity, but the T_m of this transition corresponds closely to that of the native DNA. When renatured DNA is denatured and again reannealed this also gives a product of similar T_m.

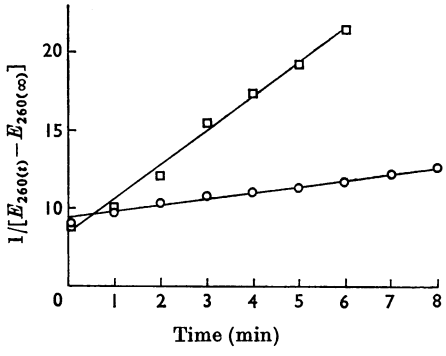

Fig. 4. Reciprocal second-order rate plot for mitochondrial DNA of *T. pyriformis* strain T. The ordinate represents the reciprocal of the remaining hyperchromicity of the renaturing DNA. $E_{260(\infty)}$ and $E_{260(t)}$ are the E_{260} values for the fully renatured DNA and for the partially renatured DNA at time t (min) during the reaction respectively. Renaturation was at 60°C in 2 × SSC (○) or in 1 M-NaCl–0.2 mM-EDTA–5 mM-tris–HCl buffer, pH 7.8 (□).

Renaturation kinetics. To investigate quantitatively the complexity of *T. pyriformis* mitochondrial DNA the kinetics of renaturation were studied. A second-order reciprocal plot of points taken from the continuous tracing on the Servoscribe recorder was made as described by Wetmur & Davidson (1968) and a straight line obtained for about 75% of the reaction (Fig. 4). No evidence of heterogeneity was observed. The DNA samples were sheared by sonication and the sedimentation coefficient in alkali was determined for each sample, since the length of the DNA fragments profoundly affects the rate of renaturation. These results are shown in Table 2. The relative values for *E. coli* DNA and bacteriophage T4 DNA in 1 M-sodium chloride agree very well with those measured by Wetmur & Davidson (1968) and Wells & Birnstiel (1969) (Table 3).

DISCUSSION

That strains of *T. pyriformis* contain one and sometimes two satellite DNA bands was shown by Suyama (1966) and is confirmed here. It seems from

Table 3. *Kinetic complexity of* T. pyriformis *mitochondrial DNA*

Complexity is calculated relative to bacteriophage T4 with an assumed value of 1.3×10^8; k_2 values are all corrected to a standard sedimentation coefficient of 4S, (k_2'), as described by Wetmur & Davidson (1968).

DNA sample	k_2'	Complexity
Bacteriophage T4	16	1.3×10^8
E. coli	1.2	1.75×10^9
T. pyriformis mitochondrial	70*	30×10^6

* Mean of values from Table 2

Table 2. *Renaturation kinetics of* T. pyriformis *mitochondrial DNA*

Kinetics were followed at (T_m − 25°C). DNA was sheared to the sedimentation coefficients shown.

DNA sample	Buffer	$S_{20,w}^{pH\ 13}$	Concn. of DNA (μg/ml)	Rate constant k_2 ($l\,mol^{-1}\,sec^{-1}$)
E. coli	2 × SSC	4	75	0.41
Bacteriophage T4	2 × SSC	4	50	5.0
T. pyriformis mitochondrial	2 × SSC	4	15	32
E. coli	1 M-Na^+*	5	50	1.5
Bacteriophage T4	1 M-Na^+*	5	50	21
T. pyriformis mitochondrial	1 M-Na^+*	4	10	74
		4	18	50
		4	13	75
		4	9	87
		12.5	17	400

* 1 M-NaCl–0.2 mM-EDTA–0.5 mM-tris–HCl buffer, pH 7.8.

our work and that of Suyama (1966) that the satellite of lower buoyant density than the main band is usually of mitochondrial origin. This is clear from our results with strain T and is suggested by the enrichment of the light satellite in mitochondrial preparations from strain W. Suyama (1966) obtained enrichment of a light satellite in mitochondrial fractions of strain GL and strains of syngens 4 and 9. He also obtained the light satellite in a pure form from mitochondria of strain ST (Suyama & Preer, 1965). In contrast, no studies have been made of the origin of the heavy satellite found in several strains of *T. pyriformis*. Suyama (1966) found three strains, GL and syngens 4 and 6, containing the heavy satellite, and we have shown strains T, W and L-II to possess a similar satellite. We have also demonstrated (R. A. Flavell & I. G. Jones, unpublished work) that the heavy satellite is present in DNA from purified nuclei of strain T. No light (i.e. mitochondrial) satellite is found in nuclear preparations.

The buoyant-density results for the mitochondrial DNA are consistent with a double-stranded DNA molecule. The increment of buoyant density ($\Delta\rho$ 0.016) is less than that for whole-cell DNA ($\Delta\rho$ 0.020) and is similar to that observed by Suyama (1966). The somewhat large increases with whole-cell DNA would be expected for a DNA of low G+C content.

The sharpness of the native mitochondrial DNA band is indicative that this DNA is homogeneous, or of high molecular weight, or both. The extremely sharp renatured band in the caesium chloride density gradient is probably a reflection of the formation of high-molecular-weight 'networks' (Britten & Kohne, 1966) formed by concatenation of DNA strands. Dawid & Wolstenholme (1968) observed an extreme increase in molecular weight of *Xenopus laevis* mitochondrial DNA on renaturation and they found sedimentation coefficients of up to 3000S for the renaturation product in their preparations. The time of renaturation of the *T. pyriformis* mitochondrial DNA (2h in 2×SSC at 60°C) is relatively long and would be expected to result in extensive network formation. The observation that mitochondrial DNA and nuclear DNA anneal separately when incubated together shows that the degree of sequence homology between these species must be low, since common compiexes could be formed by DNA types that have a sequence homology of 2–4% (Britten & Waring, 1965). This experiment would not detect a single master copy of mitochondrial DNA present in nuclear DNA; the result shown here is only relevant to general homologies. The same observations have been made by Dawid & Wolstenholme (1968) for *X. laevis* and by Sinclair *et al.* (1967) for yeast. An important consequence of this result is that trace contamination of mitochondrial DNA with nuclear DNA (less than 3% could be undetected owing to the extreme similarity in their buoyant densities) would not affect the renaturation kinetics obtained except for a small error in the estimation of mitochondrial DNA concentration.

The results of alkaline caesium chloride-density-gradient centrifugation of the mitochondrial DNA would imply the separation of the complementary strands of the native DNA (ρ 1.750 and 1.756) in the light of work with mouse satellite DNA (Flamm, McCallum & Walker, 1967), human satellite DNA (Corneo, Ginelli & Polli, 1968) and human mitochondrial DNA (Corneo, Zardi & Polli, 1968). This is a further indication of the homogeneity of mitochondrial DNA from *T. pyriformis*, and it has been suggested that this is a general property of mitochondrial DNA (Corneo *et al.* 1968).

The second-order rate constants for the renaturation of DNA allow an estimate of the kinetic complexity to be made. The work of Wetmur & Davidson (1968) and of Thrower & Peacocke (1968) suggests that the base composition of the DNA may affect the renaturation in that DNA of high G+C content renatures somewhat more rapidly than DNA of lower G+C content. The magnitude of this effect is shown by the discrepancy between the calculated complexity of *E. coli* DNA (1.75×10^9) and the analytical complexity (2.5×10^9). From inspection of the results of Wetmur & Davidson (1968) and Borst (1969) it appears that the effect becomes less pronounced at G+C contents lower than 30%. We have therefore calculated the complexity of *Tetrahymena* mitochondrial DNA relative to bacteriophage T4 DNA (Table 3). The value obtained is 30×10^6 daltons with an experimental error of about $\pm5\times10^6$. The source of the error is primarily in the difficulty in determining the sedimentation coefficients, since the k_2 values are reproducible within 5% in duplicate determinations. The uncertainty introduced by the difference of G+C content between the standard (34%) and *Tetrahymena* mitochondrial DNA (~25%) is likely to be less than 10% and therefore within the experimental error. The value of 30×10^6 for the kinetic complexity of *Tetrahymena* mitochondrial DNA is in very good agreement with the estimate of 3.4×10^7 daltons made by Suyama & Miura (1968) from electron micrographs of mitochondrial lysates of *T. pyriformis* strain ST. We are able on the basis of this result to distinguish between several models for *Tetrahymena* mitochondrial DNA. It is not compatible with a genetic complexity of 2.4×10^8 daltons (the DNA content of a single mitochondrion; Suyama & Preer, 1965) or with a complexity of 10^7 daltons as found in mammalian mitochondria. We therefore conclude that the mitochondrion of *T. pyriformis* contains about eight molecules that are

at least of extremely similar base sequence since they form common duplexes at a rate indistinguishable from the pairing of homologous complementary strands.

That the duplexes are formed by precise base-pairing is implied by the thermal stability of the renaturation product, which has a T_m indistinguishable from that of the native mitochondrial DNA.

The significance of the threefold greater informational content in the protozoan mitochondrion compared with animal mitochondrial DNA is difficult to assess owing to the paucity of data on the function of mitochondrial cistrons. Suyama & Eyer (1968) have detected mRNA synthesis in mitochondria *in vitro* and Suyama (1967) showed that the rRNA from *T. pyriformis* mitochondria hybridizes with mitochondrial DNA. One presumes that the extra 2.0×10^7 daltons of information present in *T. pyriformis* mitochondrial DNA is functional in RNA and protein synthesis. It is noteworthy that plant (*Phaseolus vulgarus* and *Phaseolus aureus*) mitochondrial DNA molecules appear to be similar in length to those found in *T. pyriformis* mitochondria. Wells & Birnstiel (1969), however, have shown that the kinetic complexity of lettuce mitochondrial DNA is 1.4×10^8, a value of the same order of magnitude as T-even phages. It is clear that the examination of other mitochondrial DNA species by this technique is needed to give further information on the variation of the complexity of mitochondrial DNA.

We thank the Science Research Council for a grant for the purchase of equipment and the Medical Research Council for a postgraduate studentship for R.A.F.

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