

Macronuclear DNA in *Stentor coeruleus*: a first approach to its characterization

BY B. PELVAT AND G. DE HALLER

Laboratoire de Biologie des Protistes,
Département de Biologie Animale, Université de Genève,
1211 Genève 4 (Suisse)

(Received 10 October 1975)

SUMMARY

The macronuclei of *Stentor coeruleus* were isolated on a discontinuous sucrose gradient and their DNA was purified by conventional methods. The GC content was 32 mole %. The DNA banded as a single peak on analytical ultracentrifugation at 1.691 g/cm³. The molecular weight of the DNA was 5×10^6 to 4×10^7 daltons. Genome size determined by DNA-DNA reassociation kinetics was 6×10^{10} daltons. The macronuclear genome was mostly simple, about 85 % being made of non-repetitive sequences.

1. INTRODUCTION

Stentor coeruleus is a common freshwater protozoan which contains two different types of nuclei: a nodular macronucleus and several micronuclei (Plate 1, fig. 1). In a general way, genetic continuity is maintained in ciliates by the micronucleus through mitosis and conjugation. In heterotrichs, micronuclear function and formation of the macronucleus is not well understood. Conjugation is observed in *Blepharisma* (Giese, 1973; Miyake & Beyer, 1973). In *Spirostomum*, conjugation is rare, but macronuclear development with well-organized chromosomes has been described by Rao (1968). Little information is available for *Stentor coeruleus*. In this species, conjugation is a very rare event (Plate 1, fig. 2) (Tartar, 1961). It sometimes appears in laboratory cultures, but no reproducible method for inducing it has been reported. It is possible that autogamy occurs, but it has not been reported to date. The macronucleus of ciliates has essentially a metabolic function. A few years ago, the macronucleus was considered simply as highly polyploid, i.e. containing numerous copies of the micronuclear genome. More recently a series of important investigations on the genetic apparatus of *Stylonychia* on one hand (Ammermann *et al.* 1974; Prescott, Murti & Bostock, 1973) and on *Tetrahymena pyriformis* and *Paramecium aurelia* on the other hand (Allen & Gibson, 1972; Yao & Gorovsky, 1974) have been reported. This ultrastructural and biochemical information led to a new concept of the genetic apparatus in ciliate protozoa. Studies on hypotrichs show that important differences exist in the molecular weight, buoyant density and thermal denaturation profiles for the micro- and macronuclear DNA. *T. pyriformis* and *P. aurelia* are two ciliates with a much simpler pattern of

macronuclear development and they do not show these molecular differences. The origin, organization and formation of the macronucleus may thus be different in the various groups of ciliates. To our knowledge, molecular characteristics of heterotrich ciliate DNA have not been studied, except for some GC characteristics in *Blepharisma* (McCarthy, 1965) and *Stentor* (Mandel & Muzyka, 1971). In contrast, timing of DNA and RNA synthesis has been extensively studied in the macronucleus of *Stentor* and *Blepharisma* during the cellular cycle and regeneration (Giese, 1973; de Terra, 1974; Gavurin & Hirshfield, 1974; Pelvat, Hyvert & de Haller, 1973). Thus characterization of the macronuclear DNA becomes necessary to understand at a molecular level the role that the macronucleus plays in morphogenetic processes. In this paper, we report some of the characteristics of the genome in *S. coeruleus*, principally the GC content, molecular-weight estimation and renaturation kinetics. We compare our results with those obtained with other free-living ciliates. Our results suggest that the genome of *S. coeruleus* is more similar to the genetic material of *T. pyriformis* and *P. aurelia* than to that of *Stylonychia* and other hypotrichs studied to date.

2. MATERIAL AND METHODS

(i) *Culture of Stentors*

The strain of *S. coeruleus* used in this study was isolated from a pond near Geneva. Clones were grown in plastic dishes (1 l capacity) in modified Carter's medium containing 0.5 mM-KCl, 2 mM-NaCl, 0.2 mM-MgCl₂, 0.5 mM-CaCl₂ and buffered at pH 7 with 0.8 mM-NaH₂PO₄. Good cultures (250 animals/ml) could be obtained when feeding the ciliates every day with *Chlorogonium elongatum* (Cambridge strain 12/2c) axenically grown in artificial medium (de Haller & Rouiller, 1961) at 18 °C with a light:dark cycle of 12:12 h. The flagellates were centrifuged and washed with Carter's medium prior to feeding the ciliates. *Stentor* with normal daylight illumination, fresh medium and a constant food supply have a generation time of 18–20 h.

(ii) *Ultrastructural studies*

Starved animals were fixed in 3% glutaraldehyde in 1/15 M phosphate buffer (PB) (Sørensen) pH 7 for 20 min, then a rinse solution of 1/15 M PB was added slowly and the cells were subsequently rinsed for three 20 min periods in rinse solution. The cells were postfixated in 2% OsO₄ in 1/15 M PB, pH 7.5 and then dehydrated in ethanol and embedded in Spurr or Epon-Araldite mixture. Thin sections were cut on a Reichert OmU2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Hitachi 7A electron microscope.

(iii) *Isolation of nuclei*

One to 2 l of ciliate culture starved for 2 days, was concentrated in a small continuous flow centrifuge and the nuclei isolated as described by Prescott *et al.* (1971) with the following modifications: concentrated cells were lysed at 0 °C in

0.01 M Tris-HCl containing 0.5% (vol./vol.) Triton X-100 and 0.004% (wt/vol.) spermidine phosphate trihydrate (pH 6.9). Triton X-100 is known to have an inhibitory effect on DNase. Stentors were left in this solution for a few minutes and the cells were broken by rapid mixing with a pipette. The nuclei were concentrated by centrifugation (5 min, 1000 g), resuspended in the same medium and 5 ml of this nuclei suspension was layered on a discontinuous sucrose gradient (15 ml 60%, 15 ml 40%, 5 ml 20%) made in a 50 ml round-bottom plastic centrifuge tube. The gradient was run for 10 min at 300 g (600 rev/min) in a MSE super minor centrifuge in a cold room at 2 °C. All the sucrose solutions contained 0.1% (vol./vol.) of Triton X-100 and 0.01% (wt/vol.) of spermidine. Macronuclei formed a grey pellet free of contaminants. Isolated macronuclei were either used immediately for preparation of DNA, or quickly frozen at -30 °C.

(iv) *Extraction and purification of DNA*

Routinely, the DNA was extracted from macronuclear preparations or whole cells by Kirby's conventional phenol method (1968) with some modifications. Macronuclei or packed cells were lysed in 2 ml of saline-EDTA (0.1 M-NaCl, 0.1 M-EDTA, pH 8 adjusted with NaOH) containing 1% SDS, and gently shaken at room temperature for 15 min in a stoppered glass tube with an equal volume of phenol saturated with saline-EDTA (pH 8). The emulsion was centrifuged 10 min at 15000 g. The aqueous layer containing the nucleic acids was removed, deproteinized once more by the same treatment and centrifuged. The aqueous phase was then treated with Sevag's solvent (chloroform/isoamyl alcohol 24:1, v/v) to complete deproteinization. The crude DNA was precipitated with 2 vols. of cold ethanol and spooled onto a glass rod. DNA was then dissolved in 0.1 × SSC (1 × SSC = 0.15 M-NaCl, 0.015 M sodium citrate, pH 7) or in Tris-EDTA buffer at pH 7 (10 mM-NaCl, 15 mM Tris, 1 mM EDTA) and treated, first with 100 µg/ml of RNase (DNase-free, Miles) and 1000 units/ml RNase T1 for 1 h at 37 °C and then with 500 µg/ml pronase (nuclease-free, Calbiochem) for 1 h at 37 °C. Subsequently, the solution was treated (usually 3 ×) with Sevag's solvent until no interphase layer was present. DNA was precipitated with cold ethanol, redissolved in 0.1 × SSC and dialysed against 0.1 × SSC if DNA was used immediately.

When little material was available and undegraded DNA was desired, another method was developed: isolated macronuclei lysed in saline-EDTA at pH 8, containing 1% SDS, was treated with RNase (150 µg/ml) for 1 h at 37 °C and subsequently for 2 h at 37 °C with pronase (1 mg/ml). The solution was shaken 2 × with Sevag's solvent and then mixed with solid CsCl to a density of 1.6 g/cm³ and centrifuged for 30 h at 35000 rev/min in a fixed-angle rotor at 20 °C. DNA was recovered from the bottom of the centrifuge tube. The purity of all DNA samples was estimated by UV absorption spectra with a Beckman 24 spectrophotometer.

(v) *Determination of thermal transition (thermal melting) of the DNA*

The general procedure was first described by Mandel & Marmur (1968) and Marmur & Doty (1962). Thermal melting being dependent on the ionic strength of

the solvent, all measurements were made in $0.1 \times \text{SSC}$ and the GC content calculated from the T_m (temp. for half denaturation) with the following equation, for this SSC dilution: $\text{GC} = (T_m - 53.9) 2.44$ (Mandel, 1968). Thermal denaturation was followed using a digital Beckman 24 spectrophotometer equipped with a Haake thermostat and coupled to an external recorder for optical density and temperature increase. The cuvettes contained 0.7 ml of DNA dissolved in 0.1 SSC at a concentration of 1 OD/ml. The rate of temperature increase was $1^\circ\text{C}/\text{min}$ during melting.

(vi) *Analytical CsCl gradient ultracentrifugation*

The method of Mandel, Schildkraut & Marmur (1968) was followed for CsCl gradient centrifugation. The DNA samples were dissolved in $0.1 \times \text{SSC}$ and adjusted to a density of 1.710 g/cm^3 with solid CsCl and centrifuged 20 h at 40 000 rev/min in a Spinco model E analytical ultracentrifuge equipped with a photoelectric scanning recording system. *M. lysodeikticus* DNA (density 1.731) was added as a reference marker for the determination of the buoyant density of the *Stentor* DNA.

(vii) *Sucrose-gradient centrifugation*

Purified DNA, dissolved in 0.1 M Tris, 0.01 M EDTA, 0.1 M-NaCl, pH 7, was layered on the top of a 5–20 % (w/v) sucrose density gradient. The tubes were chilled to 10°C and centrifuged at 25 000 rpm in a MSE HS 25 (SW rotor) for 16 h. DNA sedimentation was studied with two markers of known S value and molecular weight: *lambda* DNA (33 S) and 16 + 23 S ribosomal RNA. At the end of the run, fractions were collected from the bottom of the tube and their optical density read in a spectrophotometer at 260 nm. Determination of the molecular weight of DNA was carried out using the relationship developed by Burgi & Hershey (1963). For ^3H counting the DNA was precipitated with an equal volume of 10 % TCA, collected on Whatman GF/F glass filters, washed with cold 5 % TCA, cold 95 % ethanol and dried. Radioactivity was measured by liquid scintillation counting and cpm inserted on an absorption profile graphic.

(viii) *Chromatographic determination of the molecular weight on DEAE-cellulose*

The method of DEAE-cellulose chromatography coupled with centrifugation (Davila, Charles & Ledoux, 1965; Mingot & Davila, 1974) allows the estimation of the molecular-weight heterogeneity of DNA preparations and was used as a complementary test to sucrose gradient centrifugation. In summary, DNA samples dissolved in 0.01 M phosphate buffer, pH 7, were fixed at pH 7 to the cellulose. The sample and eluants were then placed in the upper part of a pierced tube, and passage through the cellulose facilitated by mild centrifugation (100 g for 5 min). Each eluate was collected from the lower compartment before loading with the next eluant. This technique allows selective elution of DNA bound to the matrix by modifying the type of buffer and its ionic strength: phosphate buffer with increasing NaCl molarity, followed by $\text{NH}_4\text{OH}/\text{NaCl}$ and NaOH (for details see Mingot & Davila, 1974).

(ix) *Preparation of DNA for electron microscopy*

DNA samples were prepared for electron microscopy by a modified Kleinschmidt method (Davis, Simon & Davidson, 1971; Caro, Sechaud, Boy de la Tour, personal communication, 1975). The spreading solution contained 10 μ l of DNA dissolved in Tris/EDTA buffer, 30 μ l H₂O, 10 μ l Tris 1 M-EDTA 0.1 M, 40 μ l formamide and 10 μ l cytochrome *c* (0.1%). The hypophase was twice-distilled water. The DNA film was picked up on the surface of the grid, stained with uranyl-acetate, rotary-shadowed with Pt-Pd and observed with a Hitachi 7A electron microscope. Since partial denaturation studies may provide information on the structure of DNA molecules, we examined *Stentor* DNA according to the technique used by Murti, Prescott & Pene (1972): DNA dissolved in 0.1 SSC containing 10% formaldehyde was melted in capillary tubes (15 min at 50 °C) before spreading. DNA filaments were enlarged from electron micrographs with a projector and their contour length determined with an electronic map measurer. Either *lambda* DNA or simply shadowed latex balls were used as standard. For DNA of normal base composition, molecular weight can be related to length by the following approximation: 1 micron DNA = 1.91×10^6 daltons.

(x) *Reassociation kinetic analysis (hydroxyapatite chromatography)*

The reassociation kinetics of denatured DNA was performed according to the procedure described by Britten & Kohne (1966), Wetmur & Davidson (1968) and Chrispeels (1973). Native purified DNA was sheared by sonification to 400–500 nucleotide fragments with a Branson Sonifier in 0.1 \times SSC.

The DNA was concentrated, passed through a Chelex-100 column to remove heavy metals and precipitated with 2 vols. of cold ethanol. Precipitated DNA was then dissolved in a minimum volume of 0.12 M PB. DNA was denatured in sealed glass ampoules by heating 15 min at 100 °C at different concentrations. For high DNA concentration and long incubation times, sealed capillaries were used. Heat denatured DNA was immediately incubated at 60 °C in a water bath for different times corresponding to the desired Cot. After reassociation, DNA was cooled, diluted to 2 ml with cold 0.12 M phosphate buffer and immediately applied to a hydroxyapatite column that had been equilibrated with 0.12 M PB at 60 °C. The column consisted of about 1 ml hydroxyapatite (HAP) (packed volume) supported by a disc of porous glass and maintained at 60 °C in a water-jacketed glass tube.

Non-binding single-stranded DNA was eluted with 0.12 M PB and double-stranded DNA was recovered with 0.4 M PB elution. The ratio, single-stranded/double stranded allows one to determine, for a given Cot, the percentage of reassociation for a particular DNA. The percentage of reassociation was calculated from OD measurements on the eluates recovered from 0.12 and 0.4 PB elution. *E. coli* DNA reassociation was followed according to the same system and used as standard. In some experiments, a thermal elution was used for studying stability of hybrids. Reannealed DNA was bound to HAP and the column temperature was

raised while eluting a fixed concentration of PB (0.12 M PB). As the DNA becomes thermally denatured, it elutes owing to the reduced affinity of the single-stranded for HAP.

3. ULTRASTRUCTURE OF THE MACRONUCLEUS

The vegetative macronucleus of *S. coeruleus* looks like that of other common freshwater ciliates such as *Paramecium* and *Tetrahymena* (Plate 1, fig. 3) and it is limited by an envelope consisting of two distinct unit membranes. In the nucleoplasm, small granular dense chromatin bodies, randomly scattered, ranging in size from 0.15 to 0.35 μm , are very abundant and more or less aggregated. They correspond to what is called 'small bodies' in *Paramecium* (Sonneborn, 1953; Jurand, Beale & Young, 1962).

Less-numerous larger bodies (0.61 μm) are thought to be nucleoli. Cytochemical studies have not been made but these masses are probably similar to those found in *Paramecium* (Ehret & Powers, 1955), and for heterotrichs in *Blepharisma* (Giese, 1973). *S. coeruleus* does not typically contain the large nucleoli found in another heterotrich, *Climacostomum virens* (Peck *et al.* 1975).

During division, the macronucleus of *S. coeruleus* condenses to a compact body, but never shows reorganization bands as hypotrichs do. Ultrastructural changes during morphogenetic processes have not been systematically studied in *Stentor*. Paulin (1973) reports that microtubules appear during the period of macronucleus condensation during regeneration.

4. DNA CHARACTERIZATION: RESULTS

(i) Determination of GC content

Two comparative methods were used: thermal denaturation and analytical isopycnic CsCl centrifugation (Mandel & Marmur, 1968). The hyperchromic shift of *Stentor* macronuclear DNA due to thermal denaturation in 0.1 SSC was about 35% and T_m (temperature for half denaturation) between 67 and 68 °C (Text-fig. 1). The GC content, calculated following Marmur & Doty (1962) is 31–33%. Thermal denaturation curves do not reveal heterogeneity: *Stentor* macronuclear DNA melts as a single component. Isopycnic centrifugation in CsCl with *M. lysodeikticus* DNA as reference marker ($d = 1.731 \text{ g/cm}^3$) gives, according to Schildkraut *et al.*'s relation (1962), a GC content of about 32%, for a buoyant density of 1.691. This value is in good agreement with that determined by the T_m . Text-fig. 2 shows the result of a UV scanner record for *Stentor* DNA. *Stentor* DNA shows no detectable satellite band.

(ii) Molecular-weight estimation

Three methods were used to estimate the molecular weight and heterogeneity of *Stentor* macronuclear DNA: DEAE-cellulose chromatography, sucrose-gradient centrifugation and direct measures of electron micrographs. DEAE-cellulose chromatography (Text-fig. 3) and sucrose gradient centrifugation (Text-fig. 4) indicate that the molecular weight in *Stentor* is rather high. *Lambda* DNA and 16 + 23 S-ribo-

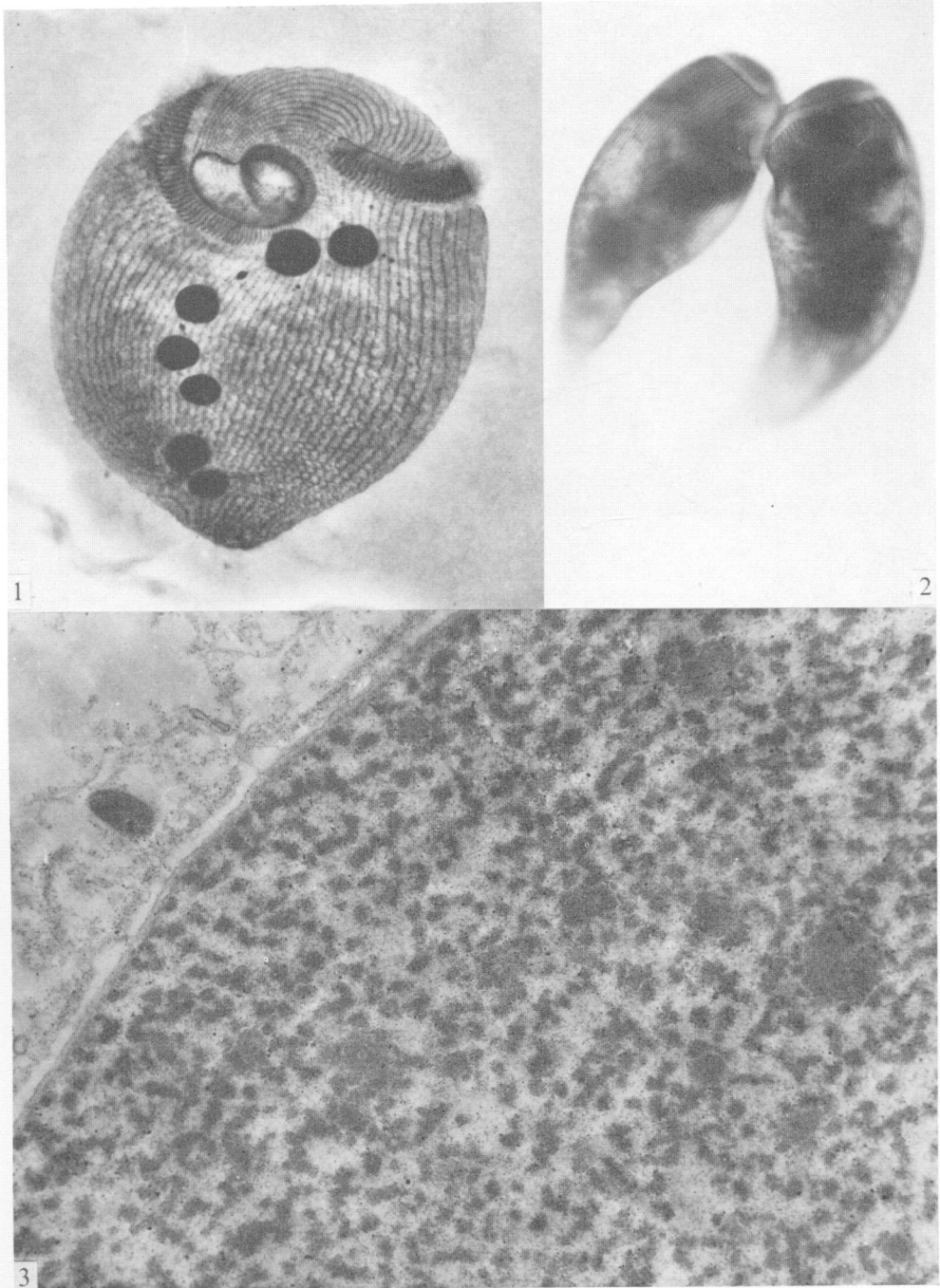


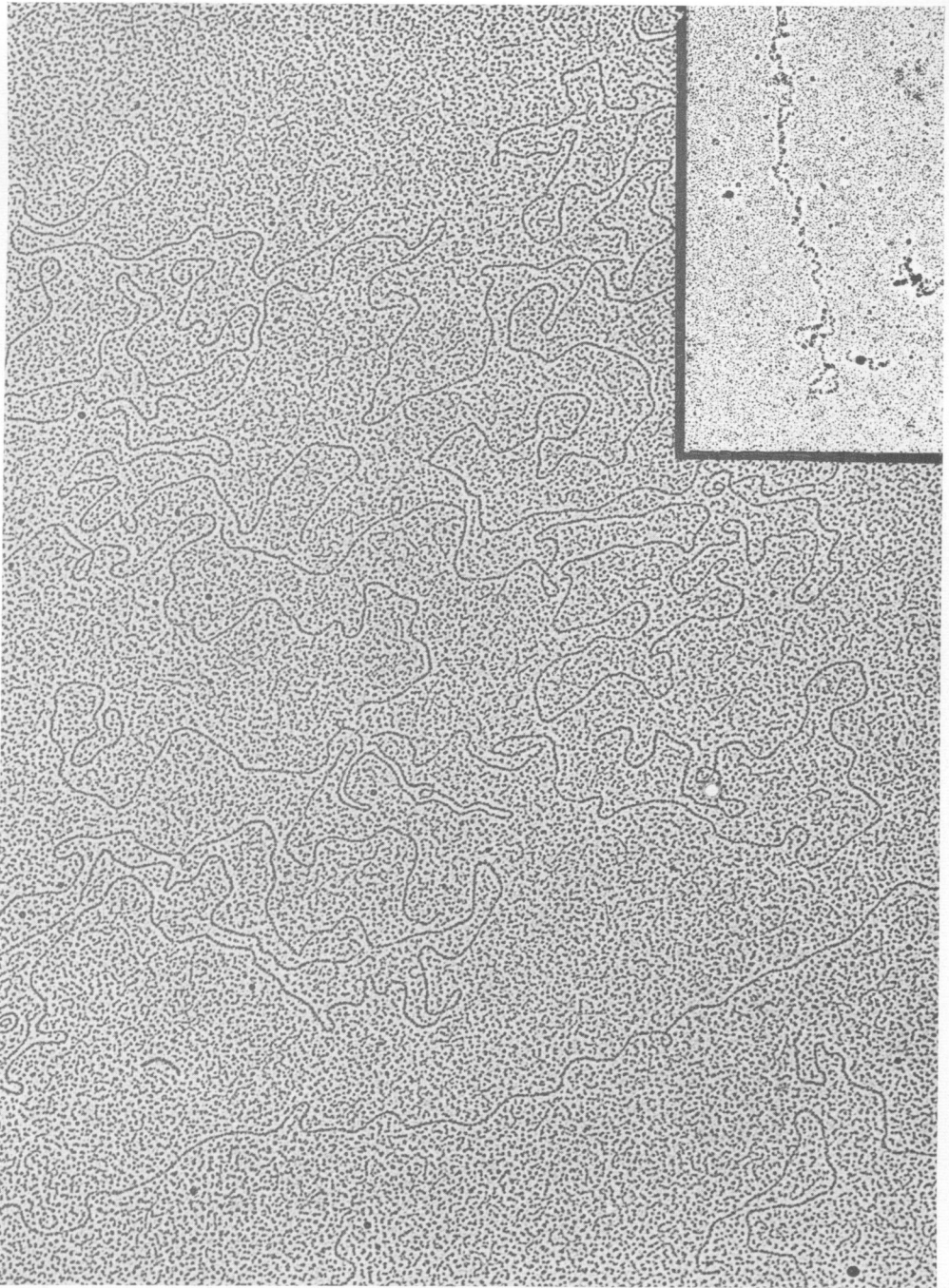
Fig. 1. *Stentor coeruleus*: protargol staining showing the nodular macronucleus.

Fig. 2. Conjugating cells.

Fig. 3. Macronucleus ultrastructure. Electron micrograph showing numerous small bodies (chromatin bodies) and larger bodies (nucleoli). $\times 10\,000$.

B. PELVAT AND G. DE HALLER

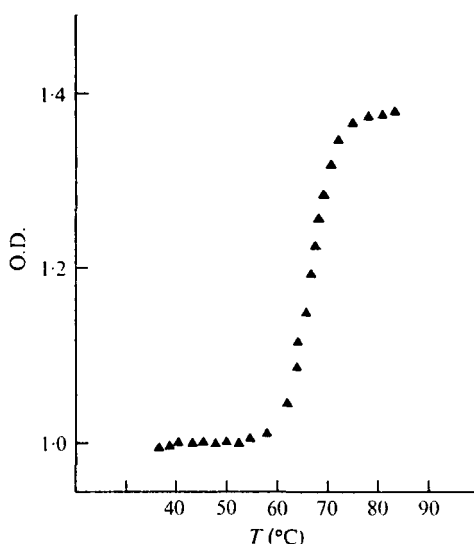
(Facing p. 282)



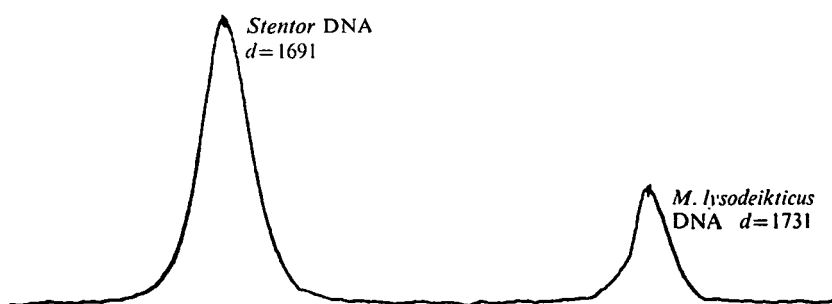
Electron micrograph of *Stentor* macronuclear DNA spread by a modified Kleinschmidt's technique and shadowed with Pt-Pd. Inset: electron micrograph of a partial denatured DNA molecule. A preliminary study showing AT-rich regions.

B. PELVAT AND G. DE HALLER

somal RNA were used as standards for sucrose-gradient centrifugation. Based upon these standards, the major part of *Stentor* DNA was estimated to be between 25 and 40S, according to the relation of Burgi & Hershey (1963), and corresponds to a molecular weight of 5×10^6 to 4×10^7 daltons. Direct measurements on electron micrographs shows molecules of variable length. We unsuccessfully tried to discriminate classes of molecules based upon length, as Ammermann *et al.* (1974) did for *Stylonychia*. However, length variation may be an artifact since shearing probably



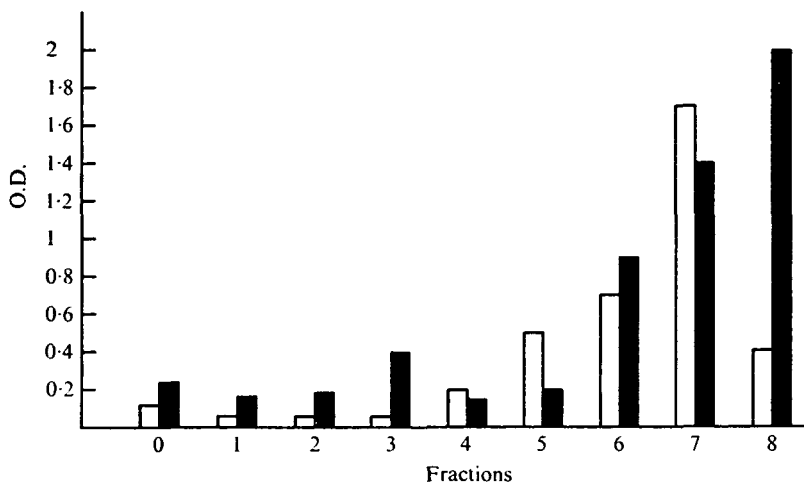
Text-fig. 1. Melting curve for macronuclear DNA in $0.1 \times \text{SSC}$. $T_m = 67^\circ\text{C}$ in 0.1 SSC .



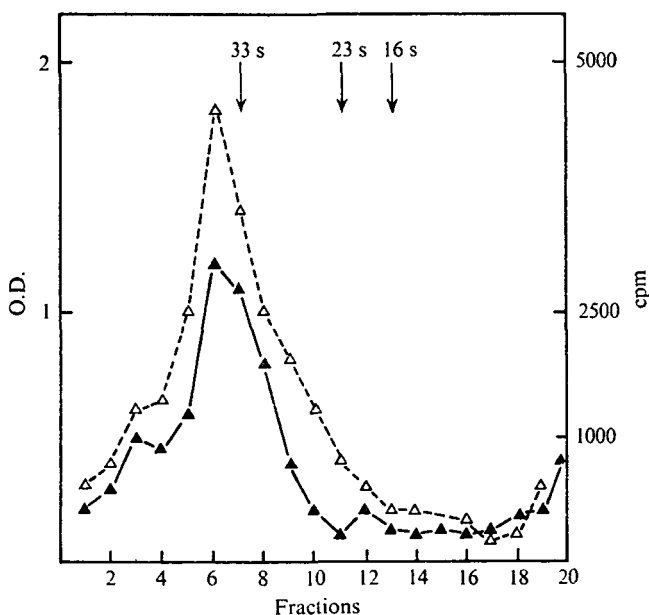
Text-fig. 2. Buoyant density profile in CsCl for *Stentor* DNA.

occurs for long molecules. The length of the majority of the molecules and particularly of the largest ones we found is nevertheless interesting. Out of 121 molecules which were measured, 52 were $15\text{--}25 \mu\text{m}$ long and correspond to a molecular weight of $3\text{--}4 \times 10^7$ daltons. These molecules probably represent much more than 50% of the total DNA. Very small (less than $3 \mu\text{m}$) and very long molecules (up to $40 \mu\text{m}$) are rarer and the largest are difficult to measure.

In *Climacostomum*, another heterotrich related to *Stentor*, we have found DNA molecules of the same order of length.



Text-fig. 3. Chromatographic profile of a *Stentor* DNA on DEAE-cellulose coupled with centrifugation. Black: *Stentor* DNA. White: salmon sperm DNA used as reference. Ordinate: optical density. Abscissa: fractions of increasing molecular weight. Fractions 0-4: m.w. 2×10^3 to 2×10^6 ; fraction 5: 2×10^6 ; fraction 6: 6×10^6 ; fraction 7: $1, 7 \times 10^7$; fraction 8: 5×10^7 and over.

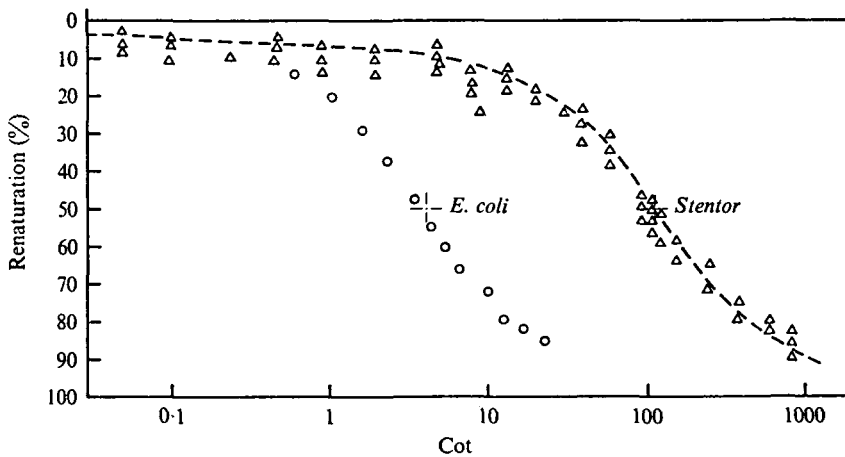


Text-fig. 4. Sedimentation profile of macronuclear DNA. The heavy fractions are on the left. ▲, Optical density; △, [³H]thymidine (cpm).

(iii) Renaturation studies

The *Stentor* macronuclear genome was investigated by methods of Britten & Kohne (1966, 1968). If the salt concentration, temperature and DNA fragment size (sonicated DNA) are kept constant, the reassociation percentage will depend on the *Cot* (concentration \times incubation time). The reassociation rate will depend on

the genomic complexity. Text-fig. 5 shows the reassociation profile of *Stentor* DNA in the form of a Cot curve. Zero-time hybridization was found to be less than 2%. This DNA which reassociates more rapidly than can be measured corresponds to DNA of unknown function, or perhaps to technical artifact. About 10–15% reanneals rapidly, indicating that the major component of this fraction is made of repeated nucleotide sequences. The remainder of the DNA (about 85%) reassociates with a second-order reaction curve, a kinetic indicating that each sequence is present only once per genome (unique sequences). However, determination of these percentages from renaturation curves may tend to overestimate



Text-fig. 5. Renaturation kinetics of *Stentor* DNA and *E. coli* DNA.

the number of unique sequences (Hough & Davidson, 1972). No heterogeneity was observed in the more slowly renaturing populations. The Cot value for unique sequences allows one to relate approximately the Cot to the haploid genome size (kinetic complexity, see Wetmur & Davidson, 1968; Britten, 1971). The size of the haploid macronuclear genome in *Stentor* was calculated with *E. coli* DNA as reference (Cot $\frac{1}{2}$ = 4, genome size = 2.7×10^9 daltons) (Cairns, 1963). By using an equation which relates Cot $\frac{1}{2}$ to genome size (Britten, 1971) we found about 6.1×10^{10} daltons per haploid genome for *Stentor* DNA (Cot $\frac{1}{2}$ = 110), which corresponds to a complexity about 20 times greater than for *E. coli* genome.

Thermal elution was used for studying the stability of hybrids. The melting of some hybrids occurs about 5 °C lower than the native DNA and most other hybrids. As possible explanation to account for the different thermal stability after renaturation, Allen & Gibson (1972) believe that not all copies present in the macronucleus are identical, or more simply, one may hypothesize that a certain degree of mismatching occurs in base pairing during reassociation.

5. DISCUSSION

(i) *GC content*

DNA base composition has become an important taxonomic criterion (Schildkraut *et al.* 1962). Generally, the percentage of GC in ciliate DNA is lower than in *E. coli* DNA (50% GC): 25–33% for *T. pyriformis* strains (Allen & Gibson, 1972), 27–30% in *P. aurelia* (Allen & Gibson, 1972; Sonneborn, 1975) and in heterotrichs, 37% in *Blepharisma* (McCarthy, 1965) and 31–33% in *S. coeruleus* (Mandel & Muzyka, 1971; and this work). The analysis of nucleic acids in free-swimming protozoa is complicated by the fact that very few of them can be cultivated axenically. Additionally, Allen & Gibson (1971) reported that differences in base ratios can be found between bacterized and axenic forms. Cummings (1972) showed that, in *P. aurelia*, the GC content measured from purified macronuclei is slightly lower (21–26%) than from whole cells (29–30%). In our case, it was interesting to see if the *Stentor* we used, from local origin, had the same composition as those studied by Mandel & Muzyka (1971), and to compare our results, obtained on isolated macronuclei, with their work on whole cells. Our results are generally in good agreement with theirs, except that we found a 1–2% lower GC content.

The DNA from many eukaryotes exhibits, in CsCl, one or more minor bands, called satellites. In ciliates, satellite DNA has been found in some strains of *P. aurelia* (Soldo & Godoy, 1972) and in *T. pyriformis* strain GL (Sueoka, 1961). Gall (1974) and Engberg *et al.* (1974) have shown that macronuclear satellite DNA of *T. pyriformis* GL corresponds to ribosomal cistrons. In another strain, *T. pyriformis* WH-6, no detectable satellites were found by Yao & Gorovsky (1974) and no marked differences between micro- and macronuclear DNA base composition was observed. In contrast, the DNA of the macronucleus of *Stylonychia* is quite homogeneous in CsCl gradients when compared to the DNA of the micronucleus (Prescott *et al.* 1973; Ammermann *et al.* 1974). *S. coeruleus* macronuclear DNA bands as a single, homogeneous component, showing no satellite bands. The ultracentrifugation curve, published by Mandel & Muzyka (1971) for *Stentor* shows no satellite either. However, in some species, satellite DNA is difficult to demonstrate, since its base composition is quite similar to that of the main band (Tobia *et al.* 1972).

(ii) *Molecular weight*

Estimation of the molecular weight of eukaryotic DNA is difficult. The content in nucleic acids is greater than in viruses and bacteria and it is almost impossible to isolate eukaryote DNA without degradation: mechanical shearing and other factors such as specific site intracellular nucleases may occur and explain, for example, the heterogeneity observed in measurements on electron micrographs. Two basically different molecular-weight classes of macronuclear DNA, according to the mode of formation of the macronucleus, have been reported in ciliates. It was thus interesting to see if *Stentor* DNA was more closely related to one class than to the other. The molecular weight of the DNA from hypotrichs is low. In *Stylonychia* Prescott *et al.* (1973) reported that all the macronuclear DNA molecules

consist of short pieces, ranging from 0.2 to 2.2 μm , whereas micronuclear DNA molecules are many times larger. The authors conclude that the DNA in the macronucleus of this ciliate may represent free genes. Very low molecular weight has also been found recently in *Oxytricha* and *Euplotes* (Wesley, 1975). *T. pyriformis* seems to have another molecular organization. Murti (cited by Raikov & Ammermann, 1974) demonstrated that the macronuclear DNA molecules are much longer in this ciliate (about 35 μm corresponding to a m.w. of about 6×10^7 daltons) than in *Stylonychia*. The molecular weight of the DNA of *T. pyriformis* GL, calculated by Miyagishi & Andoh (1973) after sucrose gradient centrifugation, is heterogeneous, ranging between 33 and 40S (3×10^7 – 10^8 daltons); however, S is affected by the form of the molecule and aggregation effects. In *P. multimicronucleatum* syn-gen 2, continuous stretches of over 80 μm (m.w. higher than 10^8) have been measured on electron micrographs (Barnett & Zadylak, 1974). *S. coeruleus* macronuclear DNA did not possess many short molecules like those observed in *Stylonychia*. Its average molecular weight of about 4×10^7 daltons (and more for the largest molecules measured on electron micrographs), allows one to place *Stentor* DNA closer to that of *Tetrahymena* than of hypotrichs.

(iii) Renaturation experiments

Analysis of the rate of reassociation provides two useful pieces of information: the relative proportion of repeated and unique sequences present in the haploid genome and the size and complexity of this genome (Britten & Kohne, 1968). In free-living protozoa, very few species have been studied to date by this technique. *Stentor* genome appears relatively simple, reiterated sequences representing only 10–15% of the total. In comparison, Flavell & Jones (1970) estimated that about 5% of the DNA of *T. pyriformis* is repetitive, and Allen & Gibson (1972) estimated, from reannealing profiles for the same species, that about 5–30% is repetitive, depending on the strain. Results from Yao & Gorovsky (1974) suggest that *T. pyriformis* DNA contains few highly repetitive sequences. Like *T. pyriformis*, *P. aurelia* DNA contains few repetitive sequences, estimated at about 15% of the total (Allen & Gibson, 1972). In *Stylonychia*, a large number of repeated sequences have been found in the micronuclei (Ammermann *et al.* 1974; Prescott *et al.* 1973). During formation of the macronucleus, the repeated sequences are preferentially lost, thus the macronucleus does not contain a measurable amount of repeated DNA.

The genome size of *Stentor* (6×10^{10} daltons) is not very large compared to that of other ciliates, being in the range reported by Allen & Gibson (1972) for *T. pyriformis* (4×10^{10} to 15×10^{10} daltons) and *P. aurelia* (about 20×10^{10} daltons).

In conclusion, by its structure and biochemical characteristics the macronucleus of *Stentor* appears closer to the model proposed for *T. pyriformis* and *P. aurelia* than to the one proposed for *Stylonychia* and other hypotrichs.

The authors are grateful to Dr H. Turler for his help in analytical ultracentrifugation, and to C. Pugin for technical assistance in electron microscopy. Expert advice and valuable sug-

gestions from Dr. M. Crippa and Dr M. Jacobs-Lorena of the Laboratory for molecular embryology are gratefully acknowledged. This work was supported by the Fonds National Suisse de la Recherche Scientifique (grant no. 3.603.71). We also thank Dr Ph. Dubois of the D.E.S. in Geneva for his help.

REFERENCES

- ALLEN, S. L. & GIBSON, I. (1971). The purification of DNA from the genomes of *Paramecium aurelia* and *Tetrahymena pyriformis*. *Journal of Protozoology* **18**, 518–525.
- ALLEN, S. L. & GIBSON, I. (1972). Genome amplification and gene expression in the ciliate macronucleus. *Biochemical Genetics* **6**, 293–313.
- AMMERMANN, D., STEINBRUECK, G., VON BERGER, L. & HENNING, W. (1974). The development of the macronucleus in the ciliates Protozoa *Stylonychia mytilus*. *Chromosoma* **45**, 401–429.
- BARNETT, A. & ZADYLAK, S. In VAN WAGTENDONK, W. J. (1974). *Paramecium: A Current Survey*. Elsevier Scientific Publishing Company.
- BRITTEN, R. J. (1971). Sequence complexity, kinetic complexity and genetic complexity. *Carnegie Institution Yearbook* **69**, 503–506.
- BRITTEN, R. J. & KOHNE, D. E. (1966). Nucleotide sequence repetition in DNA. *Carnegie Institution Yearbook* **65**, 78–125.
- BRITTEN, R. J. & KOHNE, D. E. (1968). Repeated sequences in DNA. *Science* **161**, 529–540.
- BURGI, E. & HERSHEY, A. (1963). Sedimentation rate as a measure of molecular weight of DNA. *Biophysical Journal* **3**, 309–321.
- CAIRNS, J. (1963). The chromosome of *E. coli*. *Cold Spring Harbor Symposium on Quantitative Biology* **28**, 43.
- CHRISPEELS, M. J. (1973). *Molecular Techniques and Approach in Developmental Biology*. London: Wiley-Interscience.
- CUMMINGS, D. J. (1972). Isolation and partial characterization of macro- and micronuclei from *P. aurelia*. *Journal of Cell Biology* **53**, 105–115.
- DAVILA, C., CHARLES, P. & LEDOUX, L. (1965). The chromatography of nucleic acid preparation on DEAE cellulose paper. *Journal of Chromatography* **19**, 382–395.
- DAVIS, R. W., SIMON M. & DAVIDSON, N. (1971). Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids. *Methods in Enzymology* **21B**, 413–428.
- DE HALLER, G. & ROUILLER, E. (1961). La structure fine de *Chlorogonium elongaten* Etude systématique au M. électronique. *Journal of Protozoology* **8**, 452–462.
- DE TERRA, N. (1974). Cortical control of cell division. *Science* **184**, 4163.
- EHRET, C. F. & POWERS, E. L. (1955). Macronuclear and nucleolar development in *Paramecium bursaria*. *Experimental Cell Research* **9**, 241–257.
- ENGBERG, J., NILSSON, J. R., PEARLMAN, R. E. & LEICK, V. (1974). Induction of nuclear and mitochondrial DNA replication in *Tetrahymena*. *Proceeding of the National Academy of Sciences, U.S.A.* **71**, 895–898.
- FLAVELL, R. A. & JONES, I. G. (1970). Kinetic complexity of *Tetrahymena pyriformis* nuclear desoxyribonucleic acid. *Biochemical Journal* **116**, 155–157.
- GALL, J. G. (1974). Free ribosomal RNA in the macronucleus of *Tetrahymena*. *Proceeding of the National Academy of Sciences, U.S.A.* **71**, 3078–3081.
- GAVURIN, L. & HIRSHFIELD, H. (1974). Synthesis and inhibition of macronuclear DNA in regenerating *Blepharisma*. *Journal of Experimental Zoology* **187**, 387–404.
- GHESE, A. C. (1973). *Blepharisma. The Biology of a Light-sensitive Protozoan*. Stanford University Press.
- HOUGH, B. R. & DAVIDSON, E. N. (1972). Studies on the repetitive sequence transcripts of *Xenopus*. *Journal Molecular Biology* **70**, 491–509.
- JURAND, A., BEALE, G. H. & YOUNG, M. R. (1962). Studies on the macronucleus of *Paramecium aurelia*. *Journal of Protozoology* **9**, 122–131.
- KIRBY, K. S. (1968). Isolation of nucleic acids with phenolic solvents. *Methods in Enzymology* **12b**, 87–99.
- MCCARTHY, B. (1965). In Chandra & Appel (1973). *Methoden der Molekularbiologie*. Stuttgart: G. Fischer Verlag.

- MANDEL, M. & MARMUR, J. (1968). Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12B**, 195–206.
- MANDEL, M. & MUZYKA, M. (1971). Desoxyribonucleic acids base composition of *Stentor coeruleus*. *Journal of Protozoology* **18**, 139–141.
- MANDEL, M., SCHILDKRAUT, C. L. & MARMUR, J. (1968). Use of CsCl gradient analysis for determining the guanine plus cytosine content of DNA. *Methods in Enzymology* **12B**, 184–195.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of DNA from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- MINGOT, L. & DAVILA, C. A. (1974). Chromatographic determination of the molecular weight of DNA. *Journal of Chromatography* **94**, 75–83.
- MIYAGISHI, A. & ANDOH, T. (1973). The DNA of *Tetrahymena pyriformis* GL strain: a mild method for preparation and its characterization. *Biochimica et Biophysica Acta* **299**, 507–515.
- MIYAKE, A. & BEYER, J. (1973). Cell interaction by means of soluble factors (genome) in conjugation of *Blepharisma intermedium*. *Experimental Cell Research* **76**, 1–15.
- MURTI, K. G., PRESCOTT, D. M. & PENE, J. J. (1972). DNA inciliate protozoan. III. Binding of RNA polymerase and denaturation at the ends of the low m.w. DNA in *Stylonychia*. *Journal of Molecular Biology* **68**, 413–416.
- PAULIN, J. J. (1973). Macronuclear reorganization during regeneration of oral membranelles in *Stentor coeruleus*. In *Progress in Protozoology* (abstract), 4th International Congress, UER, Clermont-Ferrand.
- PECK, R., PELVAT, B., BOLIVAR, I. & DE HALLER, G. (1975). Light and electron microscopic observations on the heterotrich ciliate *Climacostomum virens*. *Journal of Protozoology* **22**, 368–385.
- PELVAT, B., HYVEET, N. & DE HALLER, G. (1973). Synthèses d'acides nucléiques dans le macronoyau de *Stentor coeruleus*: Régénération et cycle de division. *Protistologica* **9**, 255–267.
- PRESCOTT, D. M., BOSTOCK, C. I., MURTI, K. G., LAUTH, M. R. & GAMOW, E. (1971). DNA in ciliated protozoa. I. Electron microscopic and sedimentation analysis of macro- and micro-nuclear DNA of *Stylonychia mytilus*. *Chromosoma* **34**, 355–366.
- PRESCOTT, D. M., MURTI, K. G. & BOSTOCK, C. J. (1973). Genetic apparatus of *Stylonychia*. *Nature* **242**, 576–600.
- RAIKOV, I. B. & AMMERMANN, D. (1974). The macronucleus of ciliates: Recent advances. In *Actualités Protozoologiques*, 4th International Congress, 1973 (ed. P. de Puytorac and J. Grain). UER Sciences, Clermont-Ferrand.
- RAO, M. V. (1968). Nuclear behaviour of *Spirostomum ambiguum* during conjugation with special reference to macronuclear development. *Journal of Protozoology* **15**, 748–752.
- SCHILDKRAUT, C. L., MANDEL, M., LEVISOHN, S., SMITH-SONNEBORN, J. & MARMUR, J. (1962). DNA base composition and taxonomy of some protozoan. *Nature* **196**, 795–796.
- SOLDO, A. & GODOY, G. A. (1972). The kinetic and complexity of *Paramecium* macronuclear DNA. *Journal of Protozoology* **19**, 673–678.
- SONNEBORN, T. M. (1947). Recent advances in the genetics of *Paramecium* and *Euplotes*. *Advances in Genetics* **1**, 264–358.
- SONNEBORN, T. M. (1953). Electron micrographs of ultra-thin sections of the nucleic of *P. aurelia*. *Microbiological and Genetical Bulletin* **7**, 24.
- SONNEBORN, T. M. (1975). *Paramecium aurelia*. In: *Handbook of Genetics*, vol. 2. Plenum Press.
- SUEOKA, N. (1961). Compositional correlation between DNA and protein. *Cold Spring Harbor Symposium on Quantitative Biology* **26**, 35–44.
- TARTAR, V. (1961). *The Biology of Stentor*. Oxford: Pergamon Press.
- TOBIA, A. M., BROWN, E. H., PARKER, R. Y. & SCHILDKRAUT, C. L. (1972). DNA replication in synchronized cultured mammalian cells. *Biophysica et Biochemica Acta* **277**, 256–268.
- WESLEY, R. D. (1975). Inverted repetitious sequences in the macronuclear DNA of hypotrichous ciliates. *Proceedings of the National Academy of Sciences, U.S.A.* **72**, 678–682.
- WETMUR, J. G. & DAVIDSON, N. (1968). Kinetics of renaturation of DNA. *Journal of Molecular Biology* **31**, 349–370.
- YAO, M. C. & GOROVSKY, M. A. (1974). Comparison of the sequences of macro- and micro-nuclear DNA of *Tetrahymena pyriformis*. *Chromosoma* **48**, 1–18.