ISOLATION AND PARTIAL CHARACTERIZATION OF

MACRO- AND MICRONUCLEI FROM

PARAMECIUM AURELIA

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ABSTRACT

A method was developed for the isolation of macro- and micronuclei from *Paramecium aurelia*. This method utilized ionic and nonionic detergents to rupture the intact cells, calcium ions and spermidine were employed to protect the nuclei, and the nuclei were purified by centrifugation. Macronuclei consisted of 22% DNA, 10% RNA, and 68% protein. Micronuclei were composed of 9% DNA, 11% RNA, and 80% protein. DNA from both macro- and micronuclei had a density of 1.687 g/cc in CsCl and 1.417 g/cc in Cs_2SO_4. These values corresponded to G + C content of about 23%. The RNA of macronuclei was examined by gel electrophoresis, and two high molecular weight species were identified having molecular weights of 1.3×10^6 and 2.8×10^6 daltons. Three syngens were studied, and in each case the conditions for isolation of the nuclei were the same and no differences were observed in the properties of the nuclei.

INTRODUCTION

An essential requisite for understanding the development and replication of eucaryotic organisms is the study of their nuclei. Several laboratories have directed their attention to the isolation of nuclei from a variety of organisms (1, 2, 11, 14, 22, 27). Of particular interest is the isolation of nuclei from the ciliated protozoa where nuclear dimorphism is exhibited. Here, the macronucleus exerts control over the physiological aspects of growth, and the micronucleus is involved in the transmission of genes during periods of macronuclear degeneration (see Sonneborn, 1970, for a review of the functions of the two types of nuclei in *Paramecium aurelia*). Some success in the isolation of nuclei from *Tetrahymena* has been reported (Evenson and Prescott, 1970; Gorovsky 1970; and Muramatsu, 1970), but only preliminary results have been published for *Paramecium aurelia* (Isaacks et al., 1969, and Stevenson, 1967).

In the present investigation, a method for the isolation of macro- and micronuclei from the ciliate *Paramecium aurelia* is presented. Morphological examination and the chemical composition of the two types of nuclei from three syngens (25, 26, 28) are also reported.

MATERIALS AND METHODS

Culture Methods

Stocks were obtained from Professor G. H. Beale's collection in Edinburgh. The syngens used were syngen 1 (stock 168), syngen 4 (stock 51), and syngen 13 (stock 321); these syngens were selected for use on the basis of their intersyngen enzyme variation (28), which could reflect ultimate differences in the behavior and properties of their nuclei.

Paramecium cultures (28 1) were grown on a bacterized *Klebsiella* grass infusion at 24°C (see

Sonneborn, 1950). For stock 168, the generation time was about 5 hr and the culture cleared in about 2 days; for stocks 51 and 321, the generation time was longer and it required about 3 days for the culture to clear. No differences in the isolation procedure were noted when the paramecia grew at the fast or the slow rate. The cultures were filtered through several layers of absorbent cotton wool and were centrifuged at a flow rate of 700–800 ml/min at 2300 rpm. The rotor was flushed twice with 350 ml of Dryl's solution (7), and the pellets of cells were resuspended and resedimented by centrifugation at 400 g for 5 min in pearshaped bottles in an MSE-medium oil centrifuge. I obtained 10–14 ml of packed cells from a 28 l culture.

Isolation of Macro- and Micronuclei

All of the following procedures were carried out at 0°–4°C.

For convenience, sufficient amounts of Dryl's solution (7) were added to the packed cells to make a total volume of 17.6 ml. Then 4.0 ml of a solution containing 0.033 M $CaCl_2$, 1.2 M sucrose, 0.011 M Tris, and 4.5 mg of spermidine trihydrochloride was added, and the solution was mixed by swirling. The cells were intact but were nonmotile, due primarily to the presence of sucrose. Then, 3.0 ml of 3% Nonidet P40 was added, followed by 3.0 ml of 2% sodium deoxycholate (obtained from the British Drug Houses, Ltd., Poole, Dorset, England). The final concentrations of the various reagents were: $CaCl_2$, 4.8 mM; sucrose, 0.17 M; Nonidet, 0.33% v/v; sodium deoxycholate, 0.22% w/v; spermidine trihydrochloride, 150–175 μg/ml; final pH was 7.1–7.3. After each addition, the cells were examined in a Zeiss phase-contrast microscope. Until the detergents were added, the cells were intact and appeared normal; some swelling occurred after the addition of the Nonidet and increased with the addition of the sodium deoxycholate. The macro- and micronuclei were clearly visible, and the appearance of these structures did not alter, as judged by phase microscopy with or without staining with methyl green, during any step in the procedure. Some disruption of the cells was apparent but the major alterations involved excessive enlargement, swelling, and apparent fragility of the whole cell. The suspension was then homogenized with a loosely-fitting Teflon pestle by three strokes of a Tri-R-stir homogenizer at a 3-setting. This minimal homogenization served to fracture the fragile cells, and released the intact nuclei.

An assessment of the above method of preparing nuclei from *Paramecium aurelia* is in order here. The aim in developing such a method was to obtain nuclei by the use of mild conditions. Early attempts with the use of Triton X, Tween 80, and sarkosyl,

other nonionic detergents, led to either incomplete disruption of the cell or disruption of the nuclei as well. Other workers (2, 9, 11, 14, 16, 22, 27) used similar methods and varying amounts of divalent cations and/or spermidine to protect the nuclei. I simply varied the conditions until a method, that described above, was achieved which was reproducible and yielded greater than 50% of the nuclei. In choosing the nonionic detergent, I noted that Triton X and Nonidet were the most effective in disrupting the cells; in fact, Triton X was the more effective detergent but required more calcium to protect the nuclei. Consequently, I chose Nonidet as being both effective and milder in its action. In agreement with others (see Muramatsu, 1970), I found that Ca^{++} protected the nuclei from disruption to a greater extent than did Mg^{++}. Also, in agreement with others (Evenson and Prescott, 1970; Gorovsky, 1970; and Kumar, 1970), spermidine protected the nuclei from disruption by nonionic detergents and also by the deoxycholate. The combination of Ca^{++} and spermidine allowed the use of lower concentrations of either reagent used singly; there is some indication (see Muramatsu, 1970) that concentrations of divalent cations above 5 mM can lead to excessive "hardness" of the nuclei. For the same reasons, the concentrations of both Ca^{++} and spermidine were reduced later in the purification steps (see below). Deoxycholate was utilized for two reasons: (a) much lower concentrations (by about one third) of nonionic detergents and consequently Ca^{++} could then be employed and (b) the nuclei ultimately obtained were contaminated to a lesser extent with cytoplasmic fragments.

After homogenization, the suspension containing macro- and micronuclei and cell debris was mixed 1:1 with a solution containing 2.35 M sucrose, 4.8 mM $CaCl_2$, 0.02 M NaCl, and 100 μg/ml spermidine trihydrochloride, to yield a final sucrose concentration of 1.26 M. The purpose of this step was to reduce losses at the interface of the gradient used for purification (see below). Blobel and Potter (1967) showed that cellular debris accumulated rapidly at the interface of sample and sucrose layer and prevented some nuclei from entering the sucrose layer. This was circumvented by increasing the sucrose concentration of the sample applied. Mixing with this solution also reduced the concentrations of the detergents; the Ca^{++} and spermidine were present to protect the nuclei. This suspension was then layered onto an equal volume of a solution containing 2.1 M sucrose, 3 mM $CaCl_2$, 100 μg/ml spermidine trihydrochloride, and 0.02 M NaCl and centrifuged in the SW-21 rotor of the MSE-superspeed 50–10,000 g, held at that force for 10 sec, and decelerated without braking. This brief centrifugation was sufficient to sediment most of the macronuclei to

the bottom without significant losses in the number of micronuclei. The homogenized supernatant was withdrawn from these tubes, relayered onto fresh sucrose solutions, and the micronuclei were pelleted by centrifugation for 1 hr at 40,000 g. The macronuclear preparations were reproducibly free of cellular debris; the only contamination consisted of unidentified crystals which were present in some of the homogenates. The micronuclei were contaminated by some pellicle-like material; this was removed by resuspending the pellets (see below) and then centrifuging the suspension for 20–30 sec at 700 g in the MSE-super minor centrifuge. The debris formed a compact pellet, and the suspension containing micronuclei was withdrawn with a Pasteur pipette and was then concentrated by centrifugation at 700 g for 10–15 min.

The pellets of macro- and micronuclei were resuspended in a buffer (RB) containing 9 mM NaCl, 3 mM Na_2HPO_4, 9 mM NaH_2PO_4, 3 mM $CaCl_2$, pH 6.2 for further study by either phase or electron microscopy. Routinely, we obtained 1–2 mg of micronuclei and 40–60 mg of macronuclei from a 28 1 culture.

Electron Microscopy

Pellets of macro- or micronuclei were fixed in 1% buffered osmium tetroxide, stained with 1% potassium permanganate containing 2.5% uranyl acetate, and embedded in Araldite, as described by Jurand and Selman (1970). Sections were examined in the AEI EM6B electron microscope and photographed on Ilford EM 5 plates.

Determination of DNA, RNA, and Protein

Pellets of macro- and micronuclei were resuspended in 1 ml of RB solution, and then 9 ml of 0.22 N perchloric acid (PCA) was added, in ice, to precipitate the macromolecules. After 30 min, this suspension was sedimented in the MSE-super minor centrifuge for 5 min at 800 g. The supernatant was saved for later analysis and the pellet was washed once with cold 0.20 N PCA and collected as above. The pellet was resuspended in 3 ml of 0.70 N PCA and heated for 15 min at 70°C. This suspension was centrifuged; the supernatant was saved for DNA and RNA analysis and the pellet was dissolved in Lowry A solution (see below) for protein analysis. This procedure essentially followed that described by Munro and Fleck (1966) for the optimum determination of nucleic acids.

DNA determinations were carried out with diphenylamine (Burton, 1956) and RNA with orcinol (Dische and Borenfreund, 1967). Ribose and deoxyribose, obtained from Sigma Chemical Co., (St. Louis, Mo.), were used as standards and, under

our conditions, 1 μg ribose corresponded to 9.1 μg RNA and 1 μg deoxyribose to 11.0 μg DNA.

Protein was determined by the Folin phenol reagent assay (Lowry et al., 1951), using an equal mixture of bovine serum albumin and egg white lysozyme, obtained from Sigma Chemical Co., as standards. The protein pellets were dissolved in Reagent A (2% Na_2CO_3 in 0.10 N NaOH) for 20–30 min at 23°C, and then appropriate dilutions were made for the actual analysis. Samples were analyzed in a Beckman DB spectrophotometer at 750 nm.

The final composition listed (see Table I) was the sum of the macromolecules contained in the many supernatants and pellets.

Preparation of RNA and DNA

Early attempts to isolate either high molecular weight DNA or RNA by using saline citrate or ethylenediaminetetraacetate (EDTA) solutions containing ionic or nonionic detergents (Marmur, 1961) failed. Other investigators have encountered similar problems (9, 27), and apparently such difficulties can be attributed to nuclease activity. Consequently, the method I found suitable for extraction of DNA and RNA involved the use of nuclease inhibitors (Parish and Kirby, 1966; Loening, 1969). Procedures involving the use of proteolytic enzymes were not attempted, although recently the use of pronase allowed the isolation of high molecular weight DNA from *Tetrahymena* (M. Gorovsky, personal communication). Pellets of macro- and micronuclei were homogenized at 0–5°C in medium containing 1% (w/v) of triisopropylnapthalenesulphonate, 6% (w/v) of sodium 4-aminosalicylate, 0.03 M NaCl, 0.05 M Tris, 0.010 M EDTA, and 6% (v/v) of phenol-cresol. For the RNA extractions, the EDTA was omitted. The phenol-cresol contained A. R. phenol (500 ml), redistilled *m*-cresol (70 ml), water to saturate (more than 150 ml), and 8-hydroxyquinoline

(0.5 g). The homogenates were rotated on a synchromotor at 2 sec/rev for 15 min in an equal volume of chloroform-*n*-octanol (9:1) (27). The water layer was withdrawn and the extraction was repeated twice.

DNA samples were dialyzed twice in saline citrate (20) and the density of DNA was determined in either CsCl or Cs_2SO_4 (4, 8), using either a 4-place or 2-place rotor in a Beckman Model E ultracentrifuge. In CsCl, centrifugation was carried out for 17–20 hr at 44,000 rpm ar 25°C; in Cs_2SO_4, centrifugation was carried out for 23–25 hr at 36,000 rpm. Denaturation of the DNA was achieved by heating at 100°C for 4 min in (one third) saline citrate (4), and chilling in ice. For the density determination, 0.8 ml solutions of native or denatured DNA at 2–3 μg/ml were added to solid cesium salt. Each DNA examined gave a single symmetric band in both CsCl and Cs_2SO_4. DNA from *Klebsiella* was added as a reference marker; compared with *E. coli* DNA (4, 8), *Klebsiella* DNA had a density of 1.718 g/cc in CsCl and 1.4273 g/cc in Cs_2SO_4.

Some difficulties were experienced in removing the RNA from the extracts of nucleic acid. The DNA, even after treatment with DNase, interfered with the gel electrophoretic analyses (see below). One method used was to precipitate the RNA with 3 M sodium acetate buffer, pH 6 at 0°C overnight (17). However, this procedure did not precipitate all of the RNA. A simpler procedure was to add 2 vol of ethyl alcohol at 23°C; the high molecular weight DNA precipitated within 2 min. This DNA precipitate was removed and the supernatant solution was incubated in the cold for 24–36 hr, which precipitated the RNA. This alcohol precipitation procedure was also utilized to further extract RNA from the sodium acetate supernatant mentioned above. In all cases, the RNA precipitates were freed from detergents and nonaqueous solvents by dissolving them in 0.5% (w/v) sodium lauryl sulfate, 0.15 M sodium acetate buffer, pH 6, at room temperature (17), and reprecipitating the RNA with 2 vol of ethyl alcohol at 0°C overnight. I estimated that the DNA precipitate contained no more than 10% RNA, without any preferential amounts of particular type of RNA. The RNA precipitates contained at least 85% RNA, as determined by the orcinol procedure.

Gel Electrophoresis

Electrophoresis was carried out following the procedure of Loening (17), using the Joyce-Loebl apparatus described. Polyacrylamide gels contained 2.4% acrylamide; running buffer contained 43.4 g of Sigma (Trisma) Tris, 46.8 g of $NaH_2PO_4 \cdot 2H_2O$, 3.7 g of EDTA, 20 g of sodium lauryl sulfate, 10 liters of distilled H_2O; sample buffer contained 6%

sucrose in running buffer. The gels were prerun for 1 hr at a constant current of 40 amps. Samples were added in sample buffer and run for 3.5 hr at 40 amps constant current (17).

Gels were scanned for UV absorbing material with a Joyce-Loebl UV Scanner at 265 nm.

Gels were stained, after a 2-day wash in tap water at room temperature to remove detergents, with a solution containing "Stains-all" in 50% formamide in water (Dahlberg et al. 1969). "Stains-all" is a term applied (5) to [1-ethyl-2-[3-(1-ethylnaphtho[1,2d] thiazolin-2-ylidene)-2 methylpropenyl] naphtho [1,2d] thiazolium bromide], obtained from Eastman Organic Chemicals, Eastman Kodak Co., Rochester, N. Y. A 0.10% solution of "Stains-all" in 100% formamide was diluted 1:20 with 100 ml of distilled H_2O and 90 ml of formamide. This was sufficient to stain two gels. Staining was carried out in the dark for 6–8 hr, and excess stain was removed by washing with tap water at room temperature for 2–3 days in the dark. RNA stained bluish-purple and DNA stained blue (5).

RESULTS

Morphology

Photomicrographs taken in the phase-microscope indicated that both preparations of nuclei had a high degree of purity. In Fig. 1 a, a representative field of macronuclei indicates that these nuclei were somewhat variable in size and shape, with maximum dimensions varying between 15 and 35 μ. The micronuclei are displayed in Fig. 1 b, where it can be noted that these nuclei appeared to be less variable in shape than the macronuclei, varying between 1.5 and 2.5 μ in diameter. It was difficult to obtain sharply defined pictures of micronuclei, however. These structures gave rise to diffraction rings, as can be readily observed, which obscured their dimensions. Pictures taken with the Zeiss Ultraphot II microscope showed no significant improvement. The only contamination noted in each nuclear preparation was an occasional cytoplasmic fragment and, depending on how well the cultures cleared during growth, an occasional bacterium in the micronuclear preparation. All three syngens of paramecia behaved in a similar manner and the dimensions measured for both macro- and micronuclei agreed with those previously noted *in situ* (15).

In the electron microscope, both types of nuclei appeared normal when compared with *in situ* nuclei (Jurand and Selman, 1970). Each type
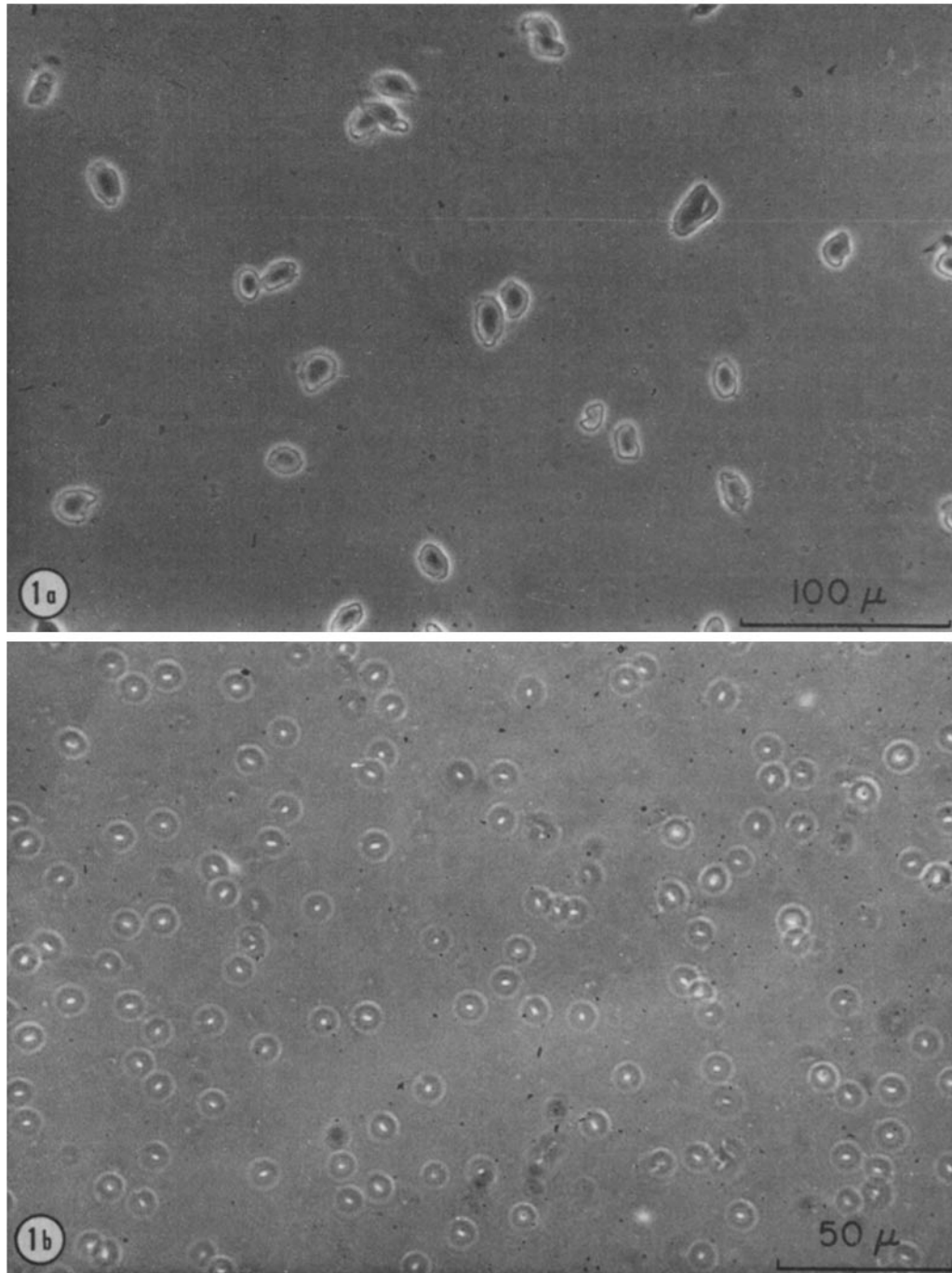
FIGURE 1   Photomicrographs of Paramecium macronuclei (Fig. 1 a) and micronuclei (Fig. b) isolated from syngen 1. The oval, white particles at the center of the ringed images are the micronuclei. Both pictures were taken with a Zeiss Phase Contrast microscope using Kodak Panatomic X film. The macronuclei were suspended in 5 ml of RB solution, and the micronuclei in 0.5 ml of RB solution. The black specks in these photographs are dust particles.
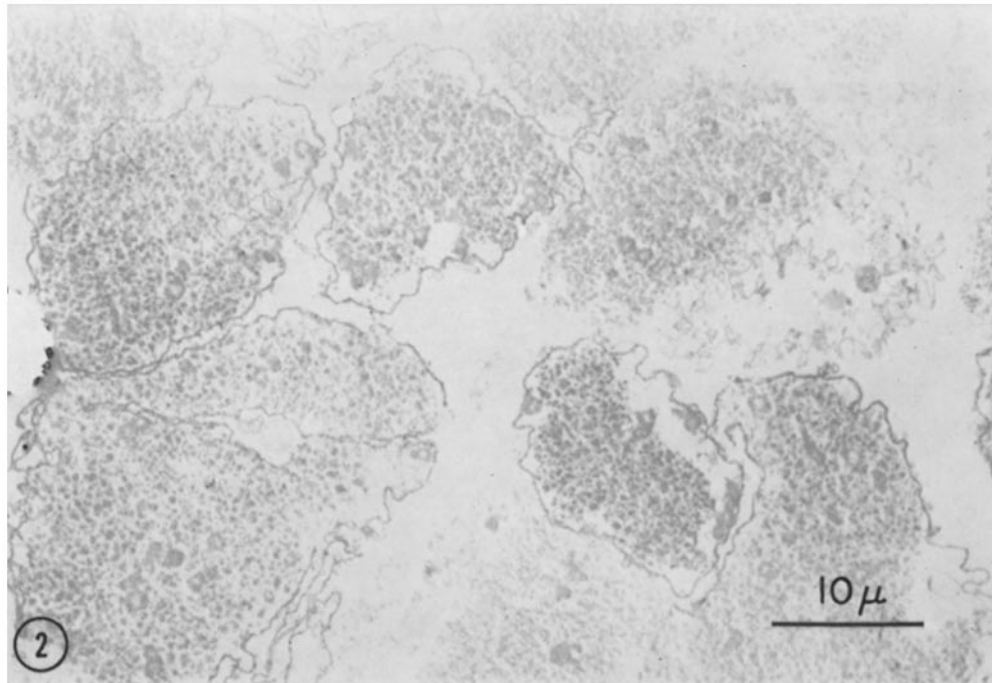
FIGURE 2 Electron micrograph of macronuclei isolated from syngen 1. Note that the nuclear membrane is present in all of these nuclei and that the nuclei are intact.

of nucleus had a well defined membrane (Figs. 2, and 3). The chromatin material in the micronuclei had a characteristic cup-shaped configuration (15), although many micronuclei had a more dispersed chromatin organization (some of these have been included in Fig. 3). It may be that these micronuclei were in some stage of mitosis or possibly differentiation to macronuclei. The chromatin material in the macronuclei consisted of two types of discrete particles, as has been well described (15). Extensive examination of these nuclei indicated that the macronuclei were contaminated only by crystals of unknown origin and composition as discussed above. The micronuclei were contaminated by some membranous fragments and trichocysts, but, very few, if any, macronuclear fragments. Examination of serial sections suggested that most of the membranous fragments were of micronuclear origin.

Composition

The amounts of DNA, RNA, and protein were determined in macro- and micronuclei, and the total of the three components was chosen as 100%. No other substance was analyzed in these nuclei.

The results are presented in Table I. The DNA/protein ratio was about 18 times greater in macronuclei and six times greater in micronuclei than for the whole cells, another indication of the purity of the preparation. These results are similar to those obtained by Bhargava and Halvorson (1971) with yeast nuclei. Each type of nucleus contained about the same percentage of RNA. The finding that the micronucleus contained significant amounts of RNA was disturbing, since other workers (13, 23) have reported that micronuclei isolated from *Tetrahymena* contained very little RNA and, using cytochemical methods, Jurand and Selman (1970) did not detect RNA in *in situ* micronuclei of *Paramecium*. As can be noted in Fig. 3, the micronuclear membranes were not sharply defined. It is possible that some cytoplasmic membranes adhered to these nuclei and carried with them cytoplasmic ribosomes. The relative constancy of the composition of micronuclei would tend to rule this out, but my finding that the isolated micronuclei of paramecium contain RNA must be verified by other techniques such as radioautography.
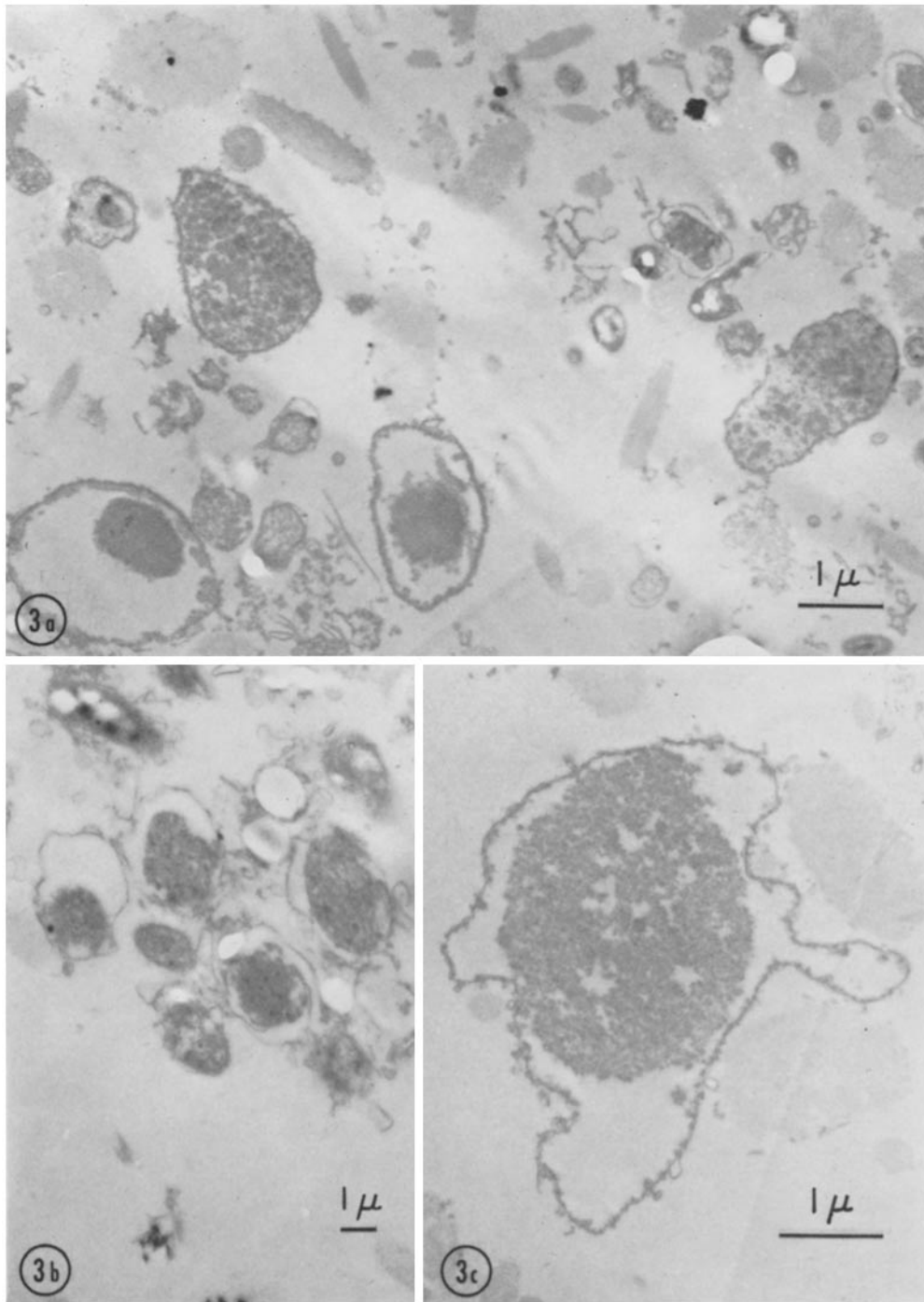
FIGURE 3   Electron micrographs of micronuclei. The nuclei were either randomly distributed (Fig. 3 a) or clumped (Fig. 3 b). Some of the nuclei had chromatin material which was dispersed in a manner similar to macronuclei (Fig. 3 c). Most of the nuclei had the usual dense chromatin material.

Density of the DNA in Cesium Salts

The DNA from macro- and micronuclei was extracted from the three syngens of paramecium, and the density was determined (Table II). It should be pointed out that the densities obtained in CsCl are in agreement with those obtained recently (10) in another stock of syngen 1, and that treatment with a proteolytic enzyme, pronase, had no effect on these densities. No significant differences were observed in the densities of DNA obtained from the three syngens examined, nor were any differences noted in the DNA isolated from macro- or micronuclei. This latter result indicates that polyploid macronuclear DNA has the same over-all base composition as the DNA contained in the diploid micronucleus. I should emphasize again that I did not detect macronuclear fragments in the micronuclear preparations, so it is unlikely that the identical densities observed with micro- and macronuclear DNA were a result of contaminated nuclei. Base compositions were calculated from data compiled by Erikson and Szybalski (1964) using densities in both CsCl and Cs_2SO_4. From CsCl studies, the G + C content of *P. aurelia* DNA was calculated to be between 22% and 26%, and from Cs_2SO_4, between 21% and 24%. There is some uncertainty in these G + C values since the standard curves (8) were subject to errors below 30% G + C content. Nevertheless, the agreement in the values calculated from either CsCl or Cs_2SO_4 densities (8) suggests that no large amounts of unusual bases are present in *Paramecium* DNA. This was also indicated by the increments in density found with the denatured DNA, 0.016 g/cc in CsCl and 0.019 g/cc in Cs_2SO_4 (8).

TABLE II
Density* of Nuclear DNA of Paramecium

Syngen	CsCl	Cs_2SO_4
	(g/cc)	(g/cc)
1 Macronuclei	1.686	1.417
Micronuclei	1.686	1.418
4 Macronuclei	1.688	1.417
Micronuclei	1.687	1.417
13 Macronuclei	1.686	1.417
Micronuclei	1.687	1.417

* Multiple analyses agreed to within <0.002 g/cc in CsCl and <0.001 g/cc in Cs_2SO_4.
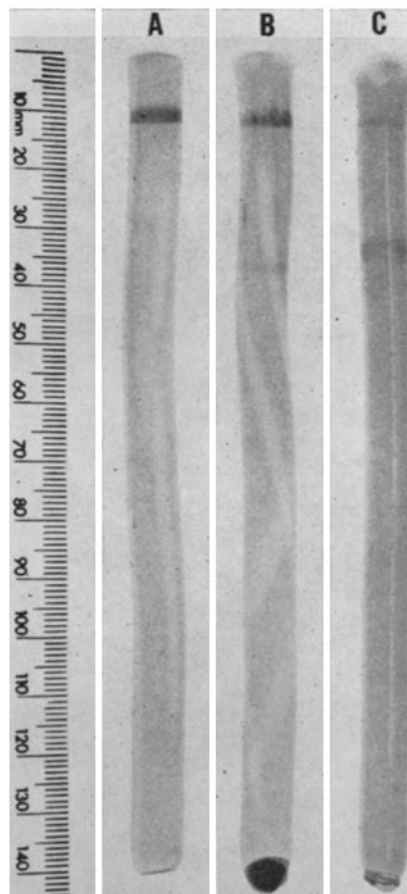

FIGURE 4 Photographs of electrophoresis gels of macronuclear RNA stained with "Stains-all". A, Syngen 13 RNA, obtained from ethyl alcohol precipitation of the sodium acetate–treated supernatants (see Materials and Methods). B, Syngen 4 RNA, obtained from sodium acetate precipitation. C, Syngen 4 RNA, another preparation of RNA obtained from sodium acetate precipitation. These gels were selected to illustrate the variability in the amounts of the types of RNA extracted, either the 2.8 × 10⁶ daltons at 11 mm or the 1.3 × 10⁶ daltons species which migrated about 35 mm. Different preparations of the same stocks exhibited varying amounts of these two types of RNA. Some variation in the distance migrated occurred from run to run, but each molecular weight was determined with marker RNA in the same gel.

Electrophoresis

Much of the recent work on the nucleic acids of eucaryotic organisms has involved the molecular weight characterization of the RNA contained in the nuclei (9, 16, 17, 18). Consequently, I

examined the RNA contained in the isolated macronuclei on acrylamide gels. In Fig. 4, it can be seen that two high molecular weight RNA species, $(1.3 \pm 0.2) \times 10^6$ and $(2.8 \pm 0.2) \times 10^6$ daltons, were detected. The stained gels illustrated were chosen to show the variability in the degree of isolation of the two species. Possibly, a third species was also present; UV scans of unstained gels indicated a peak of molecular weight $0.17 \pm 0.03 \times 10^6$ daltons near the bottom or end of the gel. Unfortunately, I was not able to stain this peak since it was lost during the washing required for the staining procedure. If it was RNA, it may have represented degradation products which arose during the isolation. Analyses of duplicate samples did indicate that the samples examined electrophoretically contained at least 85% RNA and little DNA; the presence of any DNA was readily detected in the gels by virtue of its staining properties. Characterization of the RNA contained in the micronuclei was not reproducible. Small amounts of a 1.3×10^6 daltons component and some evidence for a low molecular weight species were found but the amounts detected were insufficient to reach firm conclusions. It was clear that much greater quantities (two to three times) of micronuclei would be necessary for characterization of the micronuclear RNA by these methods.

The RNA standards used were ribosomal RNA's isolated from *Paramecium* cytoplasmic ribosomes. These RNA's have molecular weights of 1.3×10^6 and 0.70×10^6 daltons (R. Sinden, personal communication), like those in other protozoa and plants (see Loening, 1970). The 1.3×10^6 daltons standard was indistinguishable from the 1.3×10^6 daltons species found in both macro- and micronuclei.

DISCUSSION

A method for isolating macro- and micronuclei from *Parmecium aurelia* has been described. This method was reproducible and was equally successful with three different syngens of *P. aurelia*. An assessment of the different steps in the method was made earlier (see Materials and Methods), and here it is necessary only to emphasize that divalent cations and spermidine were necessary to protect the nuclei, particularly the macronucleus, from disruption. The concentrations of these protective agents were somewhat critical: if too little, the nuclei were disrupted; if too great, the cells were not quantitatively fractured, thus

preventing the liberation of the nuclei. Many methods have been reported for the isolation of nuclei from ciliated protozoa (9, 11, 14, 22, 27). Whether my method is applicable to other protozoa remains to be seen; recently, H. J. Lipps (personal communication) used this method quite successfully for the isolation of macronuclei from *Euplotes minuta*. Clearly, it is difficult to judge whether one method or protective reagent is superior to another since we have no way of determining the relationship between isolated nuclei and *in situ* nuclei. The main objective was to isolate, reproducibly, the nuclei in quantity and to then examine them; and this was achieved.

Examination of the nuclei indicated that RNA, DNA, and protein were present in substantial amounts and that the relative proportions of these macromolecules were significantly different from those found in the complete cell. My finding that the micronuclei contained significant amounts of RNA must be reexamined critically. Some consideration must be given to the possibility of contamination with bacteria or macronuclei. The culture conditions led to reduction of the bacterial concentration to less than 10^6/ml, and the harvesting methods eliminated the vast majority of these bacteria. Moreover, it was not possible to isolate bacterial ribosomal RNA by the procedures used here. With regard to macronuclear contamination, I did not observe whole or fragmented macronuclei with either phase or electron microscopy. However, these points are arguable and the micronuclei must be reinvestigated with the use of other methods, such as radioautography. The base composition of the DNA was the same in each of the syngens examined as well as in micro- and macronuclei, and no evidence for large amounts of unusual bases was observed. The low G + C content (21–26%) of *Paramecium* DNA was noteworthy, and it is obvious that further studies on this DNA are necessary. In interpreting the RNA species found by gel electrophoresis, information on the synthesis of ribosomal RNA must be mentioned. Loening (1970) has recently reviewed this area and has classified different organisms according to the molecular weights of the ribosomal RNA subunits. In this classification, protozoa and plants have the same two species, 1.3×10^6 and 0.7×10^6 daltons, whereas mammals have two species, 1.7×10^6 and 0.7×10^6 daltons. Loening (1970) further compared the sizes of the ribosomal precursor RNA molecules and listed 2.6×10^6

daltons for plants and 4.5×10^6 daltons for mammals. The sedimentation coefficient of a 2.6×10^6 daltons RNA molecule is about 36 S (18), and recently Kumar (1970) has reported that the precursor RNA molecule in *Tetrahymena* had a sedimentation coefficient of 35 S. Our finding that macronuclei from *Paramecium aurelia* contain two high molecular weight RNA species, 1.3×10^6 and 2.8×10^6 daltons, suggests that these molecules are concerned with ribosomal RNA synthesis. All of the studies summarized by Loening (1970) involved pulse labeling of the RNA, whereas mine simply involved analysis of the RNA contained in isolated nuclei. It will be of some interest to follow the course of ribosomal RNA in *Paramecium* to determine whether the stable RNA found in isolated nuclei has this origin or whether it represents messenger RNA. Labeling experiments may also reveal the presence of other RNA species, which were not detected in my studies.

For this report, I chose to study primarily the nuclear RNA and DNA and ignored the characterization of the proteins. It will, of course, be of interest to compare the nuclear proteins of paramecia with those proteins found in the nuclei of yeast (1) and *Tetrahymena* (12).

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