

A POSSIBLE SKELETAL SUBSTRUCTURE OF THE MACRONUCLEUS OF *TETRAHYMENA*

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ABSTRACT

Upon removal of chromatin from isolated macronuclei of *Tetrahymena*, residual structures are obtained, the organization of which faithfully reflects the distinctive architecture of the macronucleus. Macronuclei are isolated by a new procedure in which cells are lysed by immersion in citric acid and Triton X-100. This method is rapid and efficient and leaves the nuclear structures stripped of nuclear envelope and nucleoli. The remaining interconnected chromatin bodies are structurally differentiated into a dense outer shell and a fibrillar inner core. The fibrillar component is identified as chromatin because it is removed upon digestion with DNase and extraction with 2 M NaCl. The dense shell of the chromatin body is unaffected by the digestion procedure, which leaves a skeletal structure comprised of hollow spherical bodies. Analysis of the protein composition by SDS acrylamide gel electrophoresis before and after digestion with DNase and RNase and high-salt extraction shows that histones are diminished, whereas the nonhistone protein composition remains unchanged. It was found that DNase not only extracts chromatin but also protects the nonchromatin structure from the otherwise disruptive effects of high-salt extraction. The method used for isolating the nuclei also affects the structure remaining after the digestion procedure; the citric acid/Triton X-100 method enhances the stability of the interconnected spherical bodies. The results indicate that the method for isolating nuclei and the procedure by which chromatin is extracted are both major factors contributing to the detection of a possible nonchromatin nuclear skeleton.

KEY WORDS *Tetrahymena* · macronucleus · nuclear skeleton · nonhistone chromosomal proteins

The chromatin of the polyploid ciliate macronucleus is organized into spherical bodies of varying size, depending upon the species. In *Paramecium* and *Tetrahymena*, the chromatin bodies are ~0.1–0.2 μm in diameter. Their spherical nature was determined by whole-mount electron microscopy of isolated macronuclei disrupted on an air-water

interface (28) and by scanning electron microscopy of isolated macronuclei that had patches of nuclear envelope torn away (10). Each macronucleus contains thousands of chromatin bodies, far more than would be expected if each chromatin body were a chromosome (10). Furthermore, unlike chromosomes, the chromatin bodies are interconnected in complex arrays (10, 28). The relationship between chromosomes and chromatin bodies is unresolved.

The complex interconnection of the chromatin

bodies may be attributable to chromatin fibrils that course from one body to several others (24, 28). Alternatively, it is possible that the chromatin is embedded in a nuclear, nonchromatin structural framework or skeleton.

Recently, a number of investigators have discovered that nuclei from a variety of sources possess a skeleton of sorts that retains the overall form of the nucleus even after DNA is removed.

Chromatin-free nuclear "ghosts" were prepared from HeLa cell nuclei by Riley et al. (27). Later, it was shown that these preparations have a spongelike network, and that associated with the network are all the rapidly labeled HnRNA and some low molecular weight RNA (21).

After the enzymatic digestion of both DNA and RNA from rat liver nuclei, Bereznay and Coffey (2, 4, 5) discovered a spongelike structural matrix that retained the shape of the nucleus. Similar structures have since been found in other types of nuclei, including those of Chinese hamster cells (16), mouse liver cells (9), HeLa cells (17), and the ciliate *Tetrahymena* (14, 15, 30).

A skeletal element, called a chromosomal scaffold, has also been discovered in metaphase chromosomes freed of DNA by micrococcal nuclease digestion and of histones by dextran sulfate and heparin (1). The structure retains the size and shape of chromosomes and is composed of non-histone proteins (several major and 20–25 minor bands as determined by gel electrophoresis). Similar polypeptides have been found in interphase nuclei (1), but their structural disposition has not yet been determined.

In the course of a study of nuclear proteins and conjugation in *Tetrahymena* using macronuclei isolated by a procedure developed in this laboratory that makes use of citric acid and Triton X-100, I discovered that digestion of DNA and RNA leaves a structure the features of which faithfully reflect the unique organization of the macronucleus. This residual skeletal framework is composed of spheres organized in the same manner as in the nucleus, except that they are hollow. This residual structure differs significantly from the one reported by Herlan and Wunderlich (15), who found a spongy, fibrillar matrix after removal of DNA and RNA from the *Tetrahymena* macronucleus. I have, therefore, explored the consequences at the structural and compositional level of the citric acid/Triton X-100 isolation and the nuclease digestion procedures.

MATERIALS AND METHODS

Cell Cultures

Two strains of *Tetrahymena* were used in these studies: the amiconucleate strain GL-C of *T. pyriformis* and the micronucleate mating type III, subline B (23).

Cells were grown axenically in sterile, filtered 2% proteose peptone (Difco Laboratories, Detroit, Mich.) at 28°C without agitation. For all experiments, cells were grown to $1-2 \times 10^8$ cells/ml, which is early stationary phase under these conditions. Cells and nuclei were counted with a hemocytometer, using a phase-contrast microscope.

Isotopic Labeling

Three different labeling conditions were used: (a) cells were washed by centrifugation in 10 mM Tris, pH 7.4, and suspended in Tris. [³H]Thymidine (New England Nuclear, Boston, Mass.) was added, and cells were labeled for 1 h before further processing. (b) As in a, but cells were labeled overnight. (c) [³H]Thymidine was added to cells in culture for 24 h before collection. In c, all cells are homogeneously labeled. In a and b, the labeling is more intense, but only cells in S phase at the time of shift down (~30% of the population) are labeled (29).

Incorporation of [³H]thymidine was assayed by liquid scintillation counting of trichloroacetic acid-precipitated samples of whole cells, cell lysate, and nuclei or nuclear supernate according to the method of Byfield and Scherbaum (7). Filters used were Whatman 3 MM (Whatman, Inc., Clifton, N. J.), and the scintillation fluid used was Aquasol (New England Nuclear) or Handifluor (Mallinckrodt Inc., St. Louis, Mo.).

Nuclear Isolation

Cells were washed free of medium by centrifugation and suspended, 10-fold concentrated, in ice-cold 10 mM Tris, pH 7.4. All further operations were carried out at 4°C. An equal volume of cold 10% citric acid with 2% Triton X-100 (CT), pH 2.5, was added to the cell suspension. The turbidity of the cell suspension was instantly reduced, and examination by phase-contrast microscopy indicated that cell lysis was nearly completed by this single step. Gentle pipetting of the suspension completed the process. Nuclei were recovered by centrifuging the cell lysate at 2,000 rpm for 10 min in an IEC-CRU 5000 (Damon Corp., I. E. C. Div., Needham Heights, Mass.) refrigerated centrifuge. The nuclear pellet was resuspended in 0.5% citric acid or CMT (3 mM CaCl₂, 2 mM MgCl₂, and 20 mM Tris, pH 7.4). (Collection of macronuclei by simple centrifugation is possible because the lysis conditions do not stimulate mucocyst secretion. It is the sticky gel released by mucocyst secretion under certain lysis conditions that often necessitates sucrose gradients and results in low yields.) Suspended nuclei were centrifuged and resuspended once again to complete the removal of small contaminating particles.

Enzymatic Removal of DNA and RNA

Chromatin-free nuclear structures were prepared by the method of Herlan and Wunderlich (15). Washed nuclei were suspended in CMT containing 50 mM NaCl and incubated at 28°C for 1 h with 100 µg/ml each of DNase I or DNase II and RNase A (Worthington Biochemical Corp., Freehold, N. J.). A ninefold volume of ice-cold 2.2 M NaCl buffered with CMT was then added, and the flask was incubated on ice for 10 min. The

preparation was centrifuged at 2,000 rpm for 30 min, and the pellet was again incubated with DNase and RNase for 30 min, as described above. Chromatin-free nuclear structures were collected by centrifugation at 2,000 rpm for 10 min.

Electron Microscopy

Nuclei or chromatin-free nuclear structures were centrifuged and resuspended in 10 mM phosphate buffer, pH 7.4. An equal volume of 6% glutaraldehyde buffered with 10 mM phosphate buffer, pH 7.2, or 0.1 M cacodylate, was then added. Fixation lasted 35 min at room temperature, after which the samples were washed three times in buffer to remove the glutaraldehyde. Samples were postfixed with an equal volume of 4% OsO₄ in phosphate buffer (or 0.1 M collidine) for 2 h. They were then stained with 1% uranyl acetate for 60 min at 60°C, dehydrated stepwise in ethanol and propylene oxide, and embedded in Epon. Thin sections were stained with uranyl acetate and lead nitrate and examined with a Philips 300 EM operating at 60 kV.

SDS Gel Electrophoresis

Samples were prepared according to Laemmli (20) and were neutralized with 1 N NaOH. Polyacrylamide slab gels were prepared as described by Laemmli (20). The concentration of acrylamide in the separation gel was 12.5%, with a 5% stacking gel. Gels were stained and fixed in 10% acetic acid, 10% methanol, 0.1% aniline blue black, and 0.1% Coomassie Blue, and then destained in 10% acetic acid and 10% methanol. Molecular weight determinations were made using bovine serum albumin (68,000 daltons), ovalbumin (45,000 daltons), DNase I (31,000 daltons), and RNase A (13,000 daltons) as standards.

RESULTS

Macronuclei were isolated from *Tetrahymena* as described in Materials and Methods. Cells were washed free of medium, suspended in dilute Tris buffer, and an equal volume of CT was added. Complete cell lysis was achieved by pipetting the sample up and down a few times. This procedure leaves the nuclei intact, as is shown in Fig. 1. Because mucocyst discharge did not occur, nuclei were purified simply by low-speed centrifugation followed by resuspension in CMT or 0.5% citric acid. Nuclei purified by one such wash are shown in Fig. 2. The nuclei tend to clump together, but their form is maintained. Little material other than nuclei is present in the preparation, so cytoplasmic contamination is not a severe problem. Not only is the procedure simple and rapid, it is also efficient: recovery of nuclei in over 20 different experiments ranged from ~50 to 100%, with a typical recovery being >75%. These figures, however, are only estimates because recovered nuclei were counted using a hemocytometer, and the clumping made accurate counting difficult.

Electron microscopic examination of the isolated nuclei (Figs. 3 and 5) shows that the isolated nuclei retain their spherical shape, and that the

numerous spherical 0.1–0.2- μ m chromatin bodies of the macronucleus are still present.

However, numerous structural changes occur during isolation. These may be appreciated by a careful comparison of Figs. 4 and 5. Fig. 4 is a nucleus isolated by a more conventional procedure that uses divalent cations to stabilize the structure, and Fig. 5 is a CT-isolated nucleus.

In Fig. 4, the multiple nucleoli of the macronucleus are located in the cortex of the macronucleus, internal to the nuclear envelope. However, the entire nucleolar cortex and nuclear envelope are missing in CT-isolated nuclei (Fig. 5). Presumably, the entire nucleolar cortex is either disrupted or lifted off during the isolation procedure. What remains of the nucleus is essentially a mass of chromatin bodies. The exposure of the chromatin bodies may account for the tendency of CT-isolated nuclei to stick together to form clumps.

Three other distinctions between CT-isolated nuclei and Mg⁺⁺-isolated nuclei can be made: (a) chromatin bodies are spaced farther apart in CT-isolated nuclei; (b) nucleoplasmic material between chromatin bodies is absent in CT-isolated nuclei; and (c) the chromatin bodies of CT-isolated nuclei are considerably less electron dense.

A possible reason for the reduction in density of chromatin bodies is that the isolation procedure extracts chromatin. This, however, is not the case, as was ascertained from the following experiment:

To test whether DNA might be lost during the CT isolation procedure, 10 cm³ of cells (10⁸/ml) were labeled with [³H]thymidine (5 μ Ci/ml) for 1 h after transfer from nutrient medium to 10 mM Tris, pH 7.4. At the end of the 1-h labeling period, cells were washed, concentrated 10-fold, and lysed with CT. An aliquot was removed, and the TCA-precipitable counts per minute of the whole-cell lysate was determined. Nuclei were purified from the remainder of the whole-cell lysate, and the TCA-precipitable counts per minute in the purified nuclei was also measured.

In three experiments, the percent of counts measured for whole-cell lysates that were recovered in the purified nuclei was 76, 87, and 94%, with a mean of 86%. Since the percent of counts recovered corresponds approximately to the percent of nuclei recovered by the CT isolation procedure, it is apparent that the bulk of the DNA of the macronucleus is retained during isolation. Therefore, the reduction in the electron density of chromatin bodies after CT isolation of macronu-

clei is not the result of extensive loss of DNA but probably results from a loss of proteins.

The loss of electron density from the chromatin bodies results in a structure with a clearly differ-

entiated dense outer shell and less dense inner fibrillar contents. This is the first time that this structural differentiation of chromatin bodies has been observed.

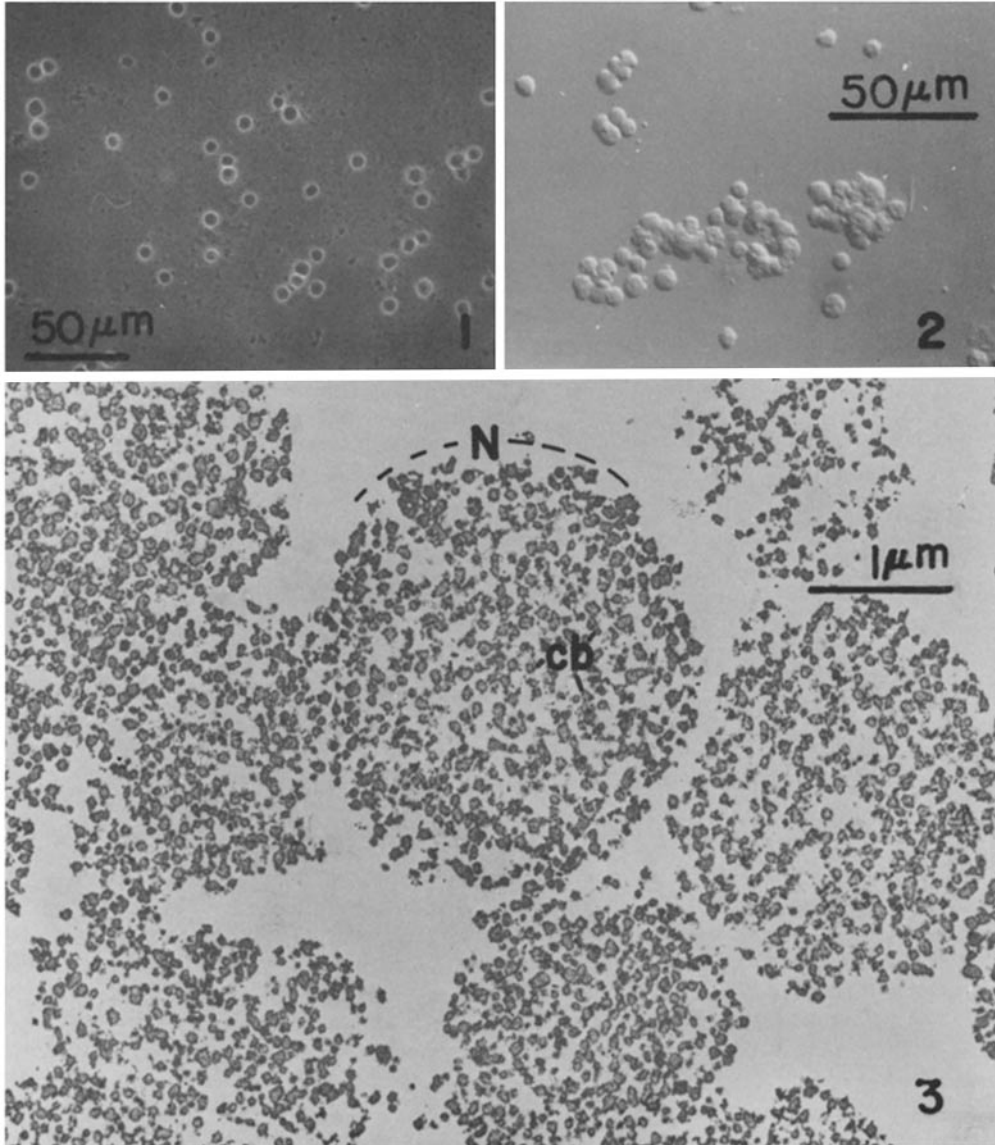
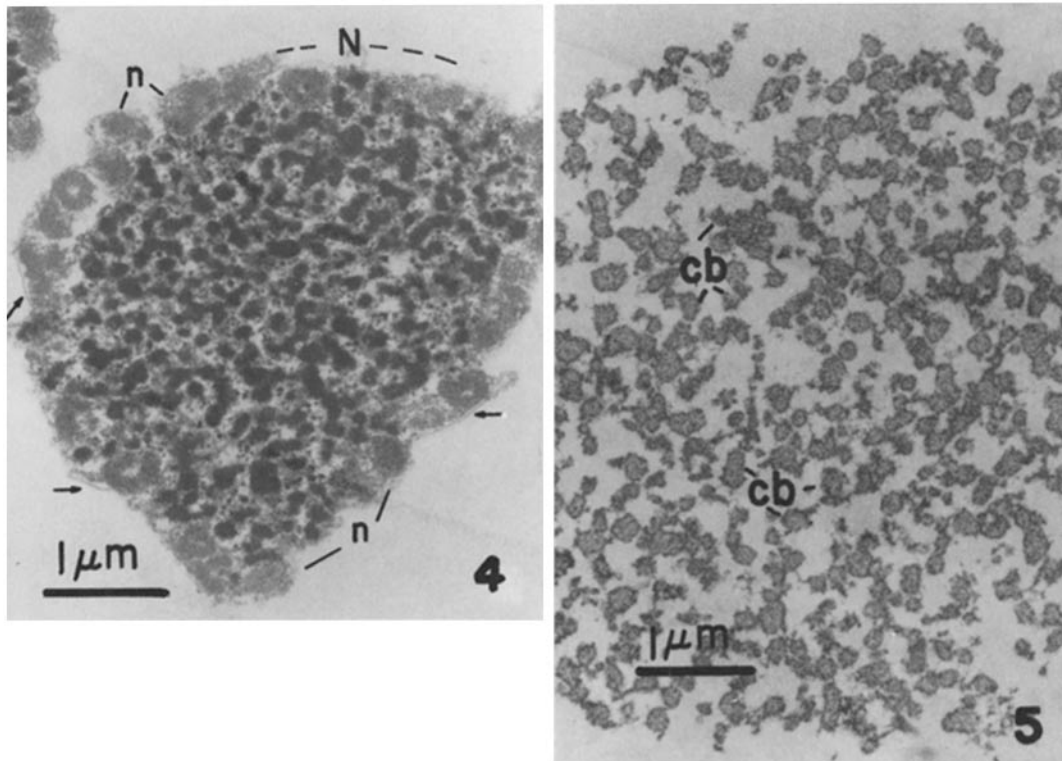


FIGURE 1 A phase-contrast micrograph of a cell lysate of *T. thermophila*. Cells of mating type BIII were suspended in CT and gently pipetted. Granular spheres are macronuclei. $\times 275$.

FIGURE 2 A Nomarski differential interference micrograph of purified macronuclei of *T. thermophila*, BIII. The nuclei are clumped together. $\times 440$.

FIGURE 3 An electron micrograph of purified *T. pyriformis* GL-C macronuclei. The nucleus (*N*) is composed of smaller units, the chromatin bodies (*cb*), which account for the granular appearance of the nuclei in Fig. 1. The purity of the preparation is indicated by the absence of structures other than nuclei. $\times 18,900$.



FIGURES 4 and 5 A comparison of macronuclei isolated by two different procedures. (Fig. 4.) A single macronucleus from GL-C isolated by lysing cells in 10 mM Tris, pH 7.4; 2 mM MgCl₂; and 1% Triton X-100. Chromatin bodies (*cb*) are dense and compact; nucleoli (*n*) are present in a cortical layer surrounding the chromatin bodies; and occasional patches of nuclear envelope are visible (arrows). $\times 16,000$. (Fig. 5) Part of a single macronucleus from GL-C isolated by lysing cells in CT. Chromatin bodies (*cb*) are less dense and more spread apart. Each chromatin body appears to have a fibrillar interior and an outer shell. Nucleoli and nuclear envelope are entirely absent. $\times 15,000$.

The DNA and RNA of CT-isolated nuclei were digested according to the procedure of Herlan and Wunderlich (15). The nuclei were incubated with DNase I and RNase A for 1 h, washed with 2 M NaCl, and reincubated in the nucleases for an additional 30 min. Surviving this treatment were spherical nuclear structures that were still intact but more refractile and smaller. Crude measurements (from micrographs) of diameters of nuclei before and after nuclease digestion indicated that the diameters of digested nuclei were reduced to about 60% of the original diameter. Thus, removal of nucleic acids results in a diminution of the size of the nucleus, an observation in agreement with that of Berezney and Coffey (5) regarding rat liver nuclei.

However, I also observed that the contraction of the macronuclei occurs immediately upon immer-

sion in the DNase I solution (even without RNase). This was not the case with DNase II, which however, by the end of the digestion process, also yielded contracted nuclei.

The extent to which DNA was removed by enzymatic digestion was determined by following the loss of counts in nuclei labeled with [³H]thymidine by three different procedures. The results comparing the effectiveness of DNase I and DNase II are presented in Table I. About 99% of the counts were removed in all cases.

Table I also shows that, at least for DNase I, >95% of the counts are lost by the end of the first digestion. Because these measurements were made before the next step, which is washing the nuclei and resuspending them in NaCl, the possibility that the counts were lost by losing nuclei during centrifugation is ruled out. I conclude that the

enzymatic treatment effectively removes the DNA from the nuclei.

From related experiments it was determined that (a) a control incubation without enzymes does not significantly remove incorporated [³H]thymidine, and that (b) incubations with DNase I alone (i.e., omitting RNase) results in the loss of >98% of the incorporated [³H]thymidine. These results indicate that the salt extraction does not remove

DNA, and that the DNase-digestion virtually frees the nuclear structure of DNA whether or not RNase is included in the incubation.

Fig. 6 shows the nuclear structure that remains after digestion with DNase and RNase. The spherical shape of the nucleus is retained, though aggregates are common. The substructure of the nucleus also appears to be intact, insofar as it comprises small spherical bodies. The spheres,

TABLE I
Loss of TCA-precipitable Counts from [³H]Thymidine-labeled, CT-isolated Nuclei

Exp.	Strain	Labeling condition*	DNase	Isolated nuclei	cpm		% loss of cpm	
					After 1st digestion	After 2nd digestion	After 1st digestion	After 2nd digestion
16-32	BIII	a	I	6.0×10^5	0.19×10^5	0.06×10^5	96.8	99.0
16-26	BIII	b	I	5.5×10^4	0.15×10^4	0.07×10^4	97.3	98.7
16-35	BIII	c	I	3.4×10^5	0.16×10^5	0.04×10^5	95.3	98.8
16-26	GL	b	I	6.4×10^4	0.13×10^4	0.07×10^4	98.0	98.9
KG, 1-2	BIII	a	II	5.3×10^4	3.5×10^4	0.10×10^4	34.0	98.1

* See Materials and Methods for a description of the labeling conditions.

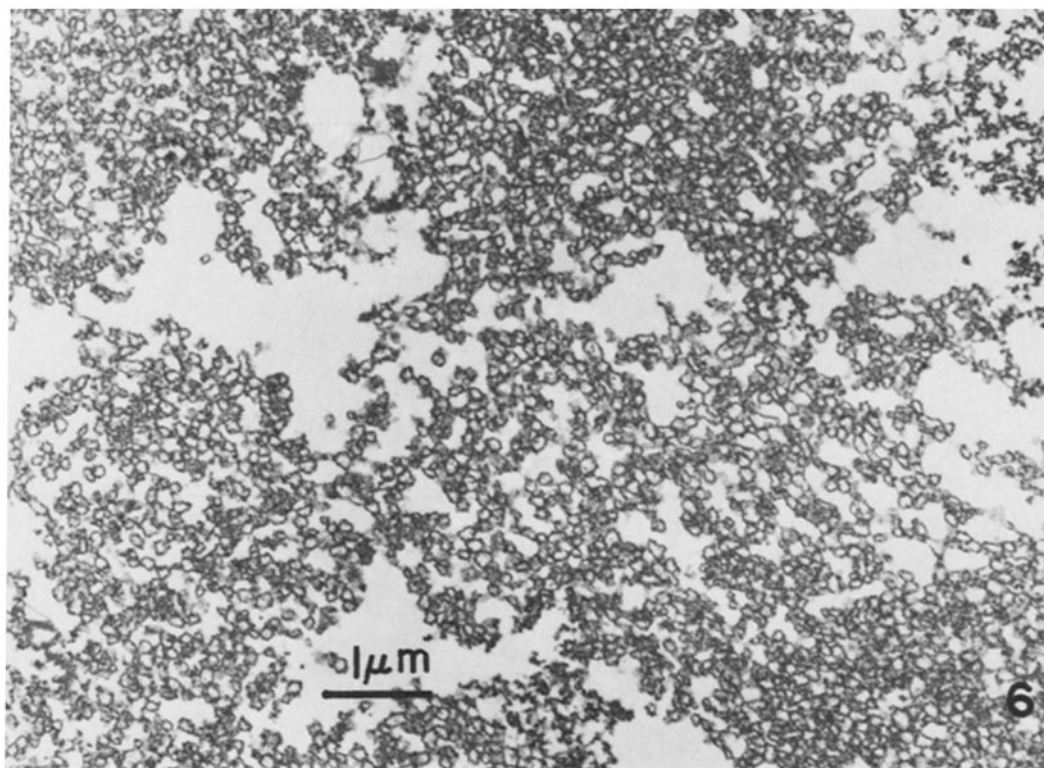


FIGURE 6 Residual nuclear structures from GL-C remaining after the nuclease digestion procedure described in Materials and Methods. The macronuclei are still composed of spherical bodies, which are now hollow. $\times 13,820$.

however, are now hollow. A comparison of the spheres before and after nuclease treatment is shown in Figs. 7 and 8.

Inasmuch as nuclease digestion results in the disappearance of the fibrillar contents of the spheres, I conclude that the fibrillar material is chromatin. Because the shells of the spheres remain intact, they represent a nonchromatin structural element. The macronucleus comprises spherical chromatin bodies; the residual nuclear structure comprises spherical chromatin-free bodies. Thus, structural plan of the skeletal remnants after nuclease digestion corresponds closely to that of the untreated macronucleus.

A higher magnification of the walls of the hollow spheres (Fig. 9) shows that they comprise globular entities measuring between 150 and 200 Å. The size of the subunits of the residual skeleton is similar to that of nucleosomes, the basic subunit of chromatin, but this is probably only a coincidence.

An attempt was made to determine the effects of the different parts of the digestion procedure on the structure of the residual nuclear body. In one experiment, RNase was omitted from the digestion procedure. At the same time phenylmethylsulfonyl fluoride (PMSF) (20 µg/ml) was added to retard proteolysis. The resulting structure (Fig. 10) resembled the hollow shell skeletal remnants obtained with the complete digestion procedure (cf. Figs. 6 and 8), confirming the interpretation that

the fibrillar contents of the spherical bodies represent chromatin. When DNase II was substituted for DNase I (with RNase included and PMSF added) a similar result was obtained: the spherical bodies were hollow, though the walls of the shells were thicker and not as well defined (Fig. 11). This indicates that the hollow shell nuclear skeleton is not a function of the particular DNase used.

Fig. 12 shows the effects of a 2 M NaCl extraction (10 min, 4°C) on the structure of the isolated

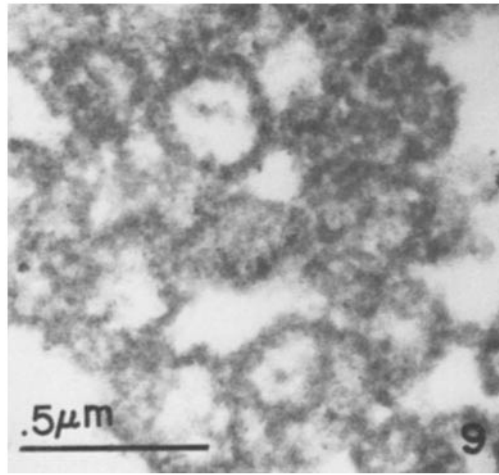
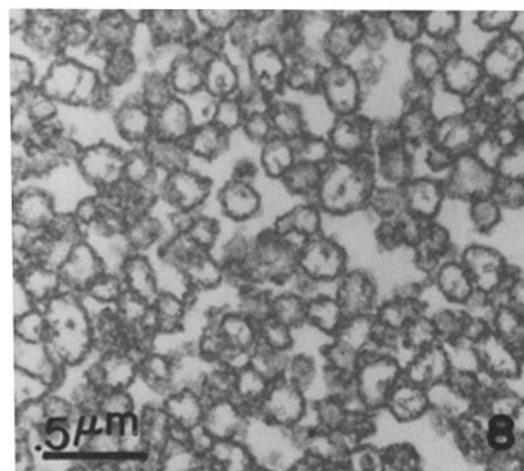
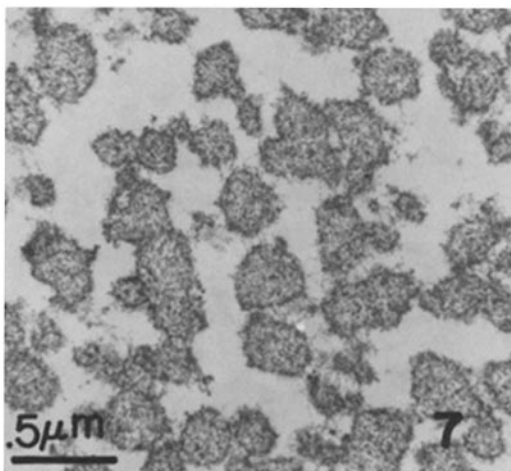
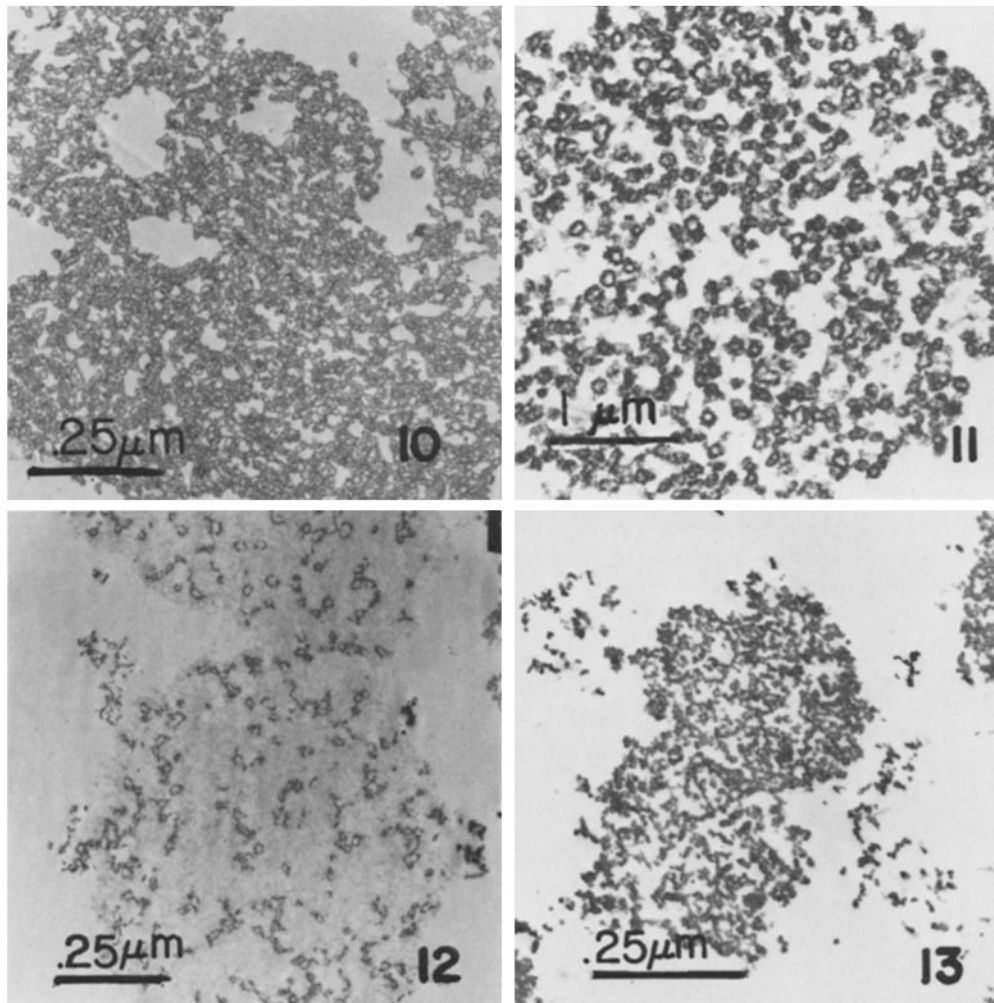


FIGURE 9 A high magnification of the walls of some BIII spheres. The walls are composed of globular units ~150–200 Å in diameter. $\times 100,000$.



FIGURES 7 and 8 A comparison of the structure of chromatin bodies from GL-C before and after routine digestion with nucleases and extraction with 2 M NaCl. (Fig. 7) Before digestion. (Fig. 8) After digestion. In contrast to Fig. 7, in Fig. 8 only the shells of the spheres are visible. The fibrillar contents are gone, the diameter of the spheres is reduced, and the spheres are more compact. $\times 27,000$.



FIGURES 10-13 CT-isolated macronuclei from BIII, treated as follows with variations of the nuclease digestion procedure: (Fig. 10) Routine digestion procedure, omitting RNase. $\times 11,760$. (Fig. 11) Routine digestion procedure, substituting DNase II for DNase I. $\times 20,000$. (Fig. 12) 2 M NaCl extraction (10 min, 4°C). $\times 6,800$. (Fig. 13) Digestion with DNase I for 60 min. $\times 8,160$.

nuclei. High-salt-extracted nuclei apparently retain their nuclear form, but the spherical-body substructure is lost. Instead, a dense network appears against a background of diffuse fibrillar material. It is important to note that a number of spherical or semispherical shapes appear as part of the dense reticulum, giving the impression that the reticulum is derived from the walls of the spherical chromatin bodies that were disrupted by the salt extraction. The fibrillar background may represent DNA released from the disrupted chromatin bodies.

These results indicate that DNase protects the

skeletal framework of the nucleus against the effects of 2 M NaCl extraction. The argument is as follows: because the hollow sphere skeleton is disrupted by 2 M NaCl in an enzyme-free incubation (Fig. 12) but not in an incubation in which DNase I and RNase (Fig. 6), DNase II and RNase (Fig. 11), or DNase I alone (Fig. 10) preceded the 2 M NaCl extraction step, it follows that the effects of the extraction by 2 M NaCl are suppressed by preincubation of the nuclei with DNase.

The appearance of nuclei treated with DNase I for 60 min (PMSF added) and fixed after washing away excess DNase (but not extracted with 2 M

NaCl) is shown in Fig. 13. It is striking that the chromatin bodies are completely electron opaque, despite the fact that, after a 1-h incubation with DNase, >95% of the DNA is removed.

Thus, DNase not only removes DNA from the chromatin granules but also increases the electron opacity of the granule spheres, induces contraction of the nucleus, and protects the walls of the spheres from 2 M NaCl extraction.

I have analyzed the protein composition of isolated, digested, salt-extracted nuclei by SDS gel electrophoresis. In the gel shown in Fig. 14, each lane was loaded with solubilized protein from $\sim 2 \times 10^6$ nuclei. Lane 1 represents control isolated nuclei not subjected to the digestion procedure. Histones stain in broad, dense bands at the bottom of the gel. In addition, about 10 nonhistone protein bands are resolved in the region between the $\sim 25,000$ - and $\sim 85,000$ -dalton range.

Lane 2 shows the protein composition of nuclei subjected solely to a 2-M NaCl extraction (10 min at 4°C). Both histone and nonhistone proteins are reduced to a level at which only a few weak bands

can be detected. Thus, high salt extracts significant amounts of protein from the macronucleus. This correlates with the ultrastructural picture, which shows that high-salt extraction disrupts the chromatin body organization of the nucleus, leaving a mass of fibrillar material with a loose reticulum of dense material (Fig. 12).

Lane 3 shows the protein composition of nuclei incubated with DNase I for 1 h, then washed free of excess DNase. These nuclei were not subjected to high-salt extraction, but PMSF was included in the DNase incubation to retard proteolysis. The banding pattern is essentially identical to that of lane 1, indicating that removal of >95% of the DNA from the macronucleus does not change its protein composition.

The banding pattern of nuclei treated with DNase I and 2 M NaCl, then DNase I again, with PMSF included in the incubation, is shown in lane 4. The most significant change, relative to the control of lane 1, is the reduction in histones. The non-histone banding pattern is essentially unchanged. Since this digestion procedure results in the chromatin-free hollow shell skeletal framework (Fig. 10), I conclude that the residual framework comprises the nonhistone proteins of the macronucleus.

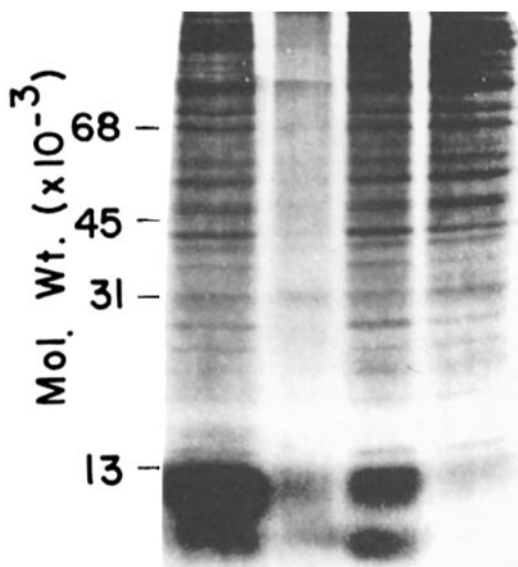


FIGURE 14 Acrylamide SDS slab gel electrophoresis with a 10–16% polyacrylamide gradient. Protein from $\sim 2 \times 10^6$ isolated macronuclei was loaded on each lane. Lane 1, control, untreated nuclei. Lane 2, nuclei extracted with 2 M NaCl (10 min 4°C). Lane 3, nuclei incubated with DNase I (with PMSF) for 60 min and washed once in CMT before fixation. Lane 4, nuclei subjected to the routine digestion procedure omitting RNase (with PMSF).

DISCUSSION

I have examined the structure and polypeptide composition of the macronucleus of *Tetrahymena* isolated by a citric acid/Triton X-100 procedure and have also analyzed the effects on the structure and composition of the macronucleus of procedures that remove chromatin by digestion with DNase and RNase and by extraction with 2 M NaCl. The results show that, after removal of chromatin, a residual proteinaceous structure remains, the organizational plan of which corresponds to that of the untreated macronucleus. This structure is perhaps analogous to the chromosomal scaffold (1) that reflects the structure of the metaphase chromosome. Also, the nonhistone polypeptide composition remains unchanged after removal of chromatin, though histones are partially extracted. These results must be interpreted in the context of the organization of the macronucleus, the method used to isolate the nuclei, and the procedure employed to remove chromatin.

The macronucleus develops, as does the micronucleus, from a diploid zygote nucleus after sexual conjugation but then undergoes extensive DNA

replication without division until it is highly polyploid. In *Tetrahymena* the macronucleus has 45 times the DNA of the micronucleus (11).

It had been thought that in ciliates the macronucleus was genetically identical to the micronucleus, both being derived ontogenetically from the same zygote nucleus. In recent years, however, examples of genetic differences between the two nuclei have been found. During the development of the macronucleus of *Stylonichia*, for example, >60% of the sequences present in the micronucleus are lost (6). The remaining sequences are present in the DNA of small, gene-sized fragments (26). Less drastic revision of the genome of *Tetrahymena* occurs, with only about 10% of the micronuclear sequences lost in the developed macronucleus (31). Ribosomal genes, however, are amplified and are present as extrachromosomal DNA fragments in hundreds of nucleoli located in the cortex of the macronucleus (32). The remainder of the DNA is in the form of long molecules in the range of 10^8 – 10^9 daltons (25).

Genetically, the macronucleus behaves as if it were composed of subunits equivalent to haploid genomes (12). From studies of the kinetic complexity of macronuclear DNA, a unit genome size of $\sim 9 \times 10^{10}$ daltons has been estimated (31). The DNA of the macronucleus, apart from rDNA, is organized into interconnected chromatin bodies, 0.1–0.2 μm in diameter.

It is possible that unit genomes are physically shared among several chromatin bodies. This is not only consistent with the calculations described above, but is supported by observations of chromatinlike fibrils connecting the spherical bodies (24, 28). Because, however, neither limited nor extensive digestion of envelope-free macronuclei isolated by two different techniques results in the dispersal of the chromatin bodies, the connecting fibrils cannot be the exclusive means by which chromatin bodies are held together. It seems that the nucleus contains a nonchromatin structural framework in which the chromatin is embedded.

In recent years, it has been demonstrated that, for a number of different eukaryotic nuclei, a structural framework remains after chromatin is removed. This structure has been variously referred to as a nuclear ghost (27), nuclear skeleton (21, 22), and nuclear protein matrix (2–4). The structural configuration of the nonchromatin residual nuclear structure is generally described as being amorphous or spongy, but until now there has been no attempt to relate the structure and the

protein composition observed to the methodology employed in isolating the nuclei and in removing the DNA or chromatin. My analysis of the residual structure of the chromatin-free macronucleus of *Tetrahymena* suggests that its configuration is very much a function of the methods used to isolate and observe it.

Herlan and Wunderlich (15, 30) described a nonchromatin structural framework from the macronucleus of *Tetrahymena* as a reversibly contractile protein matrix. They showed it to be spongy in appearance, with some traces of spherical forms in the amorphous network of material. By SDS gel electrophoresis, they determined that digestion of DNA and RNA and high-salt extraction resulted in a significant alteration of the protein composition of the nucleus.

In contrast, the nonchromatin structural framework described here is made up of hollow spherical shells, the configuration of which mirrors the organization of chromatin bodies in the macronucleus. The walls of the spheres are composed of globular subunits, unlike the fibrillar structure of the nuclear protein matrix described for liver and HeLa cell nuclei (5, 9, 13).

As determined by SDS gel electrophoresis, the protein composition of the nucleus did not change, apart from reduction in histones, after digestion with DNase and extraction with 2 M NaCl. This could represent an artefact caused by the binding of released chromatin proteins to the stable residual nuclear skeleton. Alternatively, chromatin proteins and nonchromatin structural proteins of the nucleus may be the same. The difference in protein composition of macronuclei obtained by Herlan and Wunderlich (15) after removal of chromatin might be attributable to the fact that, in this case, not only is DNA removed by the digestion, but so also are the nuclear envelope, nucleoli, and nucleoplasm. CT-isolated nuclei start with these last three components already removed. Furthermore, in other systems, differential salt extraction of nuclei results in fractions containing proteins that are similar to residual unextracted nuclear proteins (8, 19), suggesting that the same proteins may be involved in more than one kind of structural relationship.

An important finding of the present study is that high-salt treatment of isolated macronuclei quantitatively extracts a large amount of protein and destroys the subnuclear organization of the chromatin bodies. Indeed, DNase digestion of high-salt-extracted nuclei results in the complete

disintegration of the nuclear structure.¹ However, predigestion of nuclei with DNase protects the nuclei against extraction by high salts. Thus, DNase, while digesting DNA, also stabilizes the structure of the nonchromatin nuclear framework. The observed structure is, therefore, a function of the interaction between DNase and a preexisting nuclear framework.

The observed form of the nuclear skeleton must also be dependent upon the conditions under which the nuclei are isolated. A comparison of citric acid-isolated nuclei and nuclei isolated with divalent cations showed that the former contain significantly reduced amounts of protein (18). This is consistent with my morphological results, which show the CT-isolated nucleus to be stripped of nuclear envelope and nucleoli. The nucleoplasm is also absent, and the chromatin bodies are less dense than usual. A new aspect of their structure seen in this study was a thin, dense shell surrounding each body.

Direct interconnection of the shells surrounding the chromatin bodies, observed both before and after removal of chromatin, accounts for the failure of chromatin bodies to disperse upon DNase digestion. That the shells remain intact after removal of chromatin, in a form that mirrors the organization of the macronucleus, suggests that the interconnected shells represent a nuclear skeleton in which the chromatin is embedded.

However, it must be stressed that the low pH of the citric acid isolation medium might precipitate nucleoplasmic protein onto the chromatin bodies, creating a mold that remains intact after removal of chromatin. I think this unlikely as a total explanation because, even in nuclei isolated at a pH nearer 7, the chromatin bodies do not disperse upon digestion with DNase, and some spherical forms remain within the spongy matrix after removal of chromatin (15). I, therefore, think it quite possible that the CT method stabilizes a true nuclear skeleton composed of interconnected chromatin body shells. If this is correct, then it must be queried whether the spongy matrix seen in mammalian diploid nuclei after chromatin is removed might not hint at a more highly organized non-chromatin nuclear skeleton.

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¹ J. Oles. Unpublished observation.

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