THE FINE STRUCTURE OF THE NUCLEI OF *TETRAHYMENA PYRIFORMIS* THROUGHOUT THE CELL CYCLE

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ABSTRACT

The fine structure of the nuclei of logarithmically growing *Tetrahymena pyriformis*, strain HSM, was studied at 30-minute intervals throughout the cell cycle. Organisms were selected at similar stages of cytokinesis by means of a braking pipette, incubated, fixed in OsO_4, and embedded in agar to facilitate subsequent preparation for electron microscopy. Aggregates of micronuclear chromatin underwent a decrease in density and number with a concomitant increase in size throughout interphase. There were no impressive changes in macronuclear morphology. It was found possible to estimate a cell's progress through interphase by observation of micronuclear morphology, but attempts to correlate changes in fine structure with periods of DNA synthesis were unsuccessful.

INTRODUCTION

Studies of cell growth and reproduction have, until recent years, emphasized the events immediately surrounding cell division. The realization that interphase contains many events, *e.g.* DNA synthesis, concerned with cell growth and duplication, has led to many chemical and physiological investigations. The ciliated protozoan, *Tetrahymena pyriformis*, has been the object of many of these studies, largely because it may be cultured axenically in the laboratory under well controlled conditions (see reviews by Corliss (1), Holz (2), and Prescott (3)). Investigations of morphological changes during interphase, however, have been few, particularly with the electron microscope. Light microscope studies of *T. pyriformis* morphology have concentrated mainly on division (4–6) or the morphogenesis of cortical structures (7–10). Changes in nuclear morphology during interphase were not reported.

Roth and Minick (11) observed differences in the ultrastructure of "interphase" *versus* dividing cells in an amicronucleate strain (W) of *T. pyriformis*. Changes were noted in chromatin condensation, nucleoli, and nuclear granularity, as well as in mitochondria and the endoplasmic reticulum. Comparable differences between "interphase" and division were absent in a micronucleate strain (HAM 3).

Elliott *et al.* (12) studied macronuclear changes in *T. pyriformis* (strain WH_6) during and following synchronization of division by repetitive heat shocks. Their observations were interpreted as follows. "RNA bodies" (oval, membrane-less structures, entirely digested by RNase) appeared in the center of the macronucleus, moved to the periphery of the nucleus, and disintegrated. The peripherally placed nucleoli (partially digested by RNase) moved into nuclear "blebs," which pinched off into the cytoplasm. It was suggested that this might represent the morphological basis

for the movement of RNA from the nucleus to the cytoplasm which has been detected by chemical means.

Other investigators, working with *Paramecia*, have reported changes in nuclear morphology during interphase. Ehret and Haller (13) noted variations in the aggregation of nucleoli and in the texture of the nucleoplasmic matrix of *Paramecium bursaria* during the 40 to 50 minutes preceding divisions, and Jurand *et al.* (14) described the changing structure of nuclear "large bodies" (the "nucleoli" of other workers) throughout interphase in *Paramecium aurelia*. These observations were not correlated with biochemical events.

The present study was undertaken 1) to determine whether the fine structure of the nuclei of logarithmically growing *Tetrahymena* undergoes changes during interphase which can be correlated with known biochemical events of the cell cycle, and 2) to compare the observations made on heat-synchronized cells by Elliott *et al.* with the nuclear events in logarithmically growing cells.

For orientation, a brief summary of important nuclear events in *Tetrahymena pyriformis*, strain HSM, follows. The organisms contain a highly polyploid macronucleus which elongates and divides amitotically at the time of cell division. The macronuclear DNA synthesis period is during early to middle interphase (15). The micronucleus lies in a depression in the macronucleus during most of the cell cycle. Late in interphase it moves away from the macronucleus, enlarges, elongates, and divides mitotically (2, 4). Breakdown of the nuclear membrane and spindle formation, however, have not been observed (23). The micronucleus enters DNA synthesis almost immediately after it has divided, while the macronucleus is dividing and the cell is undergoing cytokinesis (16). About the time DNA synthesis is concluded, the micronucleus returns to a depression of the macronucleus. Fig. 1 illustrates the temporal relationships of macronuclear and micronuclear events.

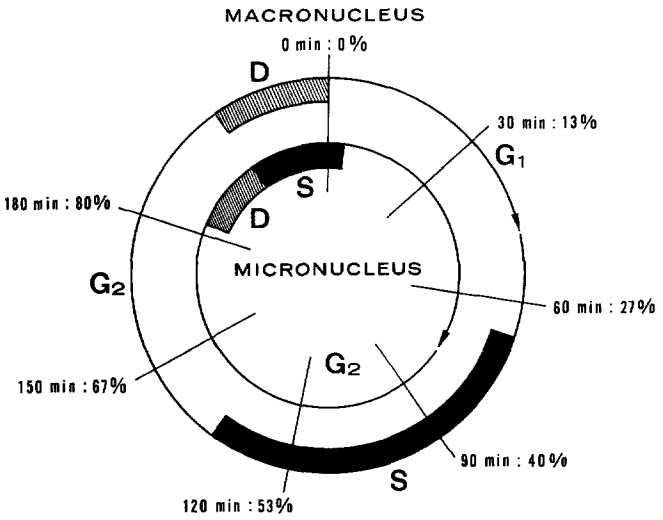


FIGURE 1 Diagram of the cell cycle of *Tetrahymena pyriformis*, strain HSM. The cycle proceeds clockwise. The outer circle represents macronuclear events; the inner circle depicts micronuclear events (16). The times of samples are shown in minutes and per cent completion of the cell cycle.

MATERIALS AND METHODS

Stock cultures of *Tetrahymena pyriformis*, strain HSM, were maintained at 29°C in synthetic medium (17) supplemented with 0.04 per cent w/v proteose peptone. Organisms in logarithmic growth were obtained by subculturing 18 to 24 hours prior to an experiment. The mean generation time of strain HSM under these conditions is 225 minutes (15).

Cells in an early stage of cytokinesis were selected over a 10-minute period by means of a braking micropipette (18). Two individuals working simultaneously were able to obtain a total of 200 to 400 cells at a similar stage of fission. The time at which 50 per cent of the cells was judged to have completed fission was designated time zero of the cell cycle. The cells were incubated at 29°C in 0.1 to 0.2 cc of medium in a 10-ml capillary bottom centrifuge tube for 0, 30, 60, 90, 120, 150, 180, or 210 minutes. These times correspond to 13, 27, 40, 53, 67, and 80 per cent completion of the cell cycle. Fixation was carried out for 1 hour at room temperature by addi-

tion of 1 cc of 1 per cent osmium tetroxide containing 45 mg sucrose per ml, buffered with veronal-acetate to pH 7.3.

The cells were embedded in a small amount of agar to facilitate their subsequent handling. This was accomplished by means of a technique described in detail elsewhere (19) and which will be presented only in outline here. The material was collected by centrifugation, rinsed with 50 per cent ethanol, centrifuged again, and transferred in 0.05 cc rinse *via* syringe and needle to a capillary tube partially filled with 2 per cent agar. After centrifugation of the cells to the surface of the agar, additional molten 2 per cent agar was added to embed the cells. The agar cylinder was pushed from the tube, flooded with molten agar on a glass slide, and a small cube of agar containing the cells was cut out. The cube was then transferred to a small vial for dehydration.

The cells in agar were dehydrated rapidly in a graded series of alcohols, cleared with propylene oxide, and embedded in Epon according to the method of Luft (20). Sections showing silver-to-pale-gold interference colors were cut on a Huxley microtome with glass knives and mounted on copper grids coated with 0.25 per cent Parlodion reinforced with carbon. The preparations were stained with saturated uranyl acetate in water for 20 minutes at 35°C, and with lead citrate (21), and examined in a Phillips EM-100B microscope at 60 kv and an original magnification of 1500 to 15,000 times.

RESULTS

The nuclei of cells fixed at time 0 are seen in various stages of division, requiring more extensive description and analysis than nuclei at other times in the cell cycle. Therefore, an orderly description of the nuclear events occurring throughout one complete cell cycle is facilitated by beginning with cells fixed at 30 minutes (13 per cent completion of the cell cycle) and progressing to dividing cells at time 0.

Micronucleus

The micronucleus at 30 minutes (13 per cent completion of the cell cycle) (Fig. 2) presents a circular profile, approximately 2 to 3 μ in diameter, surrounded by a double membrane. It contains many small clumps of electron opaque material, interpreted as chromatin, uniformly distributed throughout a less dense, finely granular nucleoplasm. No structures morphologically identifiable as nucleoli are present. The micronucleus is situated in a depression in the macronucleus (Fig. 12).

The most marked change in the micronucleus as interphase proceeds is in the pattern of chromatin material (Figs. 2 to 7). From 30 minutes (13 per cent cell cycle) to 150 minutes (67 per cent cell cycle) (Figs. 2 to 6), there is a gradual and progressive diminution in the number and density of clumps of chromatin, with a concomitant increase in their size, until by 150 minutes chromatin and nucleoplasm have similar densities. By 180 minutes (80 per cent cell cycle) (Fig. 7), in most cases, the micronucleus is observed to have begun to move away from the macronucleus and to have increased in size to about 3 to 4 μ. The chromatin material appears to have increased in density, as compared with that of cells fixed at 150 minutes (Fig. 6), and is more clearly delineated from the surrounding nucleoplasm. By 210 minutes, the micronucleus is farther away from the macronucleus, frequently appears elongated, similar to that in Fig. 8, and often displays discrete elongated clumps of chromatin comparable in density to that observed at 180 minutes. Some cells at 210 minutes have completed micronuclear division and are seen in varying stages of macronuclear division and cytokinesis.

The micronuclei shown in Figs. 2 to 7 are representative of the majority of micronuclei seen in each sample. Repetition of the experiments in each instance provided results comparable to those illustrated. Approximately 10 per cent of the nuclei resembled more closely the majority of nuclei in the preceding or following sample. This is accounted for by minor variations in progress through cytokinesis at the time of selection of cells, and intrinsic variability of the length of the cycle among individual cells. Although the synchrony of a sample is almost complete at the time that the dividing cells are selected, the synchrony deteriorates over the cell cycle. One hundred per cent of cells selected in this way enter DNA synthesis within a 15-minute period (D. M. Prescott and G. E. Stone, personal communication), but in a strain with an average generation time of 111 minutes it takes 35 minutes for 90 per cent of the cells to complete the next division (22).

In cells fixed at 0 time, micronuclei are observed in several states. A peripherally located micronucleus may appear elongated, as in Fig. 8. In some cases, a micronucleus lying at the periphery of the cell and separated from the macronucleus is seen with a circular profile and a chromatin

pattern either 1) similar to that observed in elongated dividing forms (Fig. 9), or 2) composed of many small dense clumps similar to that observed at 30 minutes (Fig. 10). In many cases, a micronucleus with the latter chromatin pattern is also observed in a depression in the macronucleus at this time (Fig. 11). It appears, therefore, that Figs. 7 to 11 illustrate a sequence in which the micronucleus moves away from the macronucleus, enlarges (Fig. 7), displays condensed chromosomes, elongates (Fig. 8), and divides without breakdown of the nuclear envelope or detectable spindle formation. These events are followed by dispersion of chromatin into many small dense clumps (Fig. 10) and return of the micronucleus to the depression in the macronucleus (Fig. 11). The latter, then, represents a very early stage in the succeeding cell cycle.

Macronucleus

The macronucleus at 30 minutes presents roughly a circular or oval profile except in sections containing a micronucleus where it has the characteristic indentation or depression (Fig. 12). It is surrounded by a double membrane and contains many evenly distributed clumps of electron-opaque material identified as chromatin by Elliott *et al.* (12) on the basis of digestibility by DNase. Many larger electron-opaque bodies presumed to be nucleoli (11, 12) are concentrated peripherally. These bodies are composed of a dense granular outer shell and a less dense inner core. The outer shell is often discontinuous, producing a crescent shape. It may be noted, however, that Jurand *et al.* (14), who studied similar bodies in the macronucleus of *Paramecium aurelia* with enzyme digestion and cytochemical methods, concluded that the large bodies contained DNA surrounded by RNA and that the small bodies contained neither DNA nor RNA.

Changes in the macronuclear morphology throughout interphase are not impressive (Figs. 12 to 17). There appears to be some decrease in density of the chromatin bodies at 150 minutes (Fig. 16), the time of least density in micronuclear chromatin. In over 200 micrographs of macronuclei, no structures resembling the RNA bodies described by Elliott *et al.* for heat-shocked cells were observed. Although occasionally some irregularity of the macronuclear profile is noted (*e.g.*, Fig. 15), nuclear "blebs" comparable to those described for heat-shocked cells were never

Key to Labeling

M,	macronucleus	*c*,	chromatin
mi,	micronucleus	*n*,	nucleolus
Mae,	macronuclear envelope	*m*,	mitochondrion
mie,	micronuclear envelope	*cf*,	cleavage furrow

FIGURES 2 to 7 The micronucleus at 30-minute intervals throughout interphase, illustrating the progressive decrease in number and density and increase in size of chromatin bodies up to 150 minutes, followed by the appearance of discrete clumps of chromatin (? chromosomes) at 180 minutes.

FIGURE 2 Micronucleus at 30 minutes (13 per cent completion of the cell cycle). × 19,000.

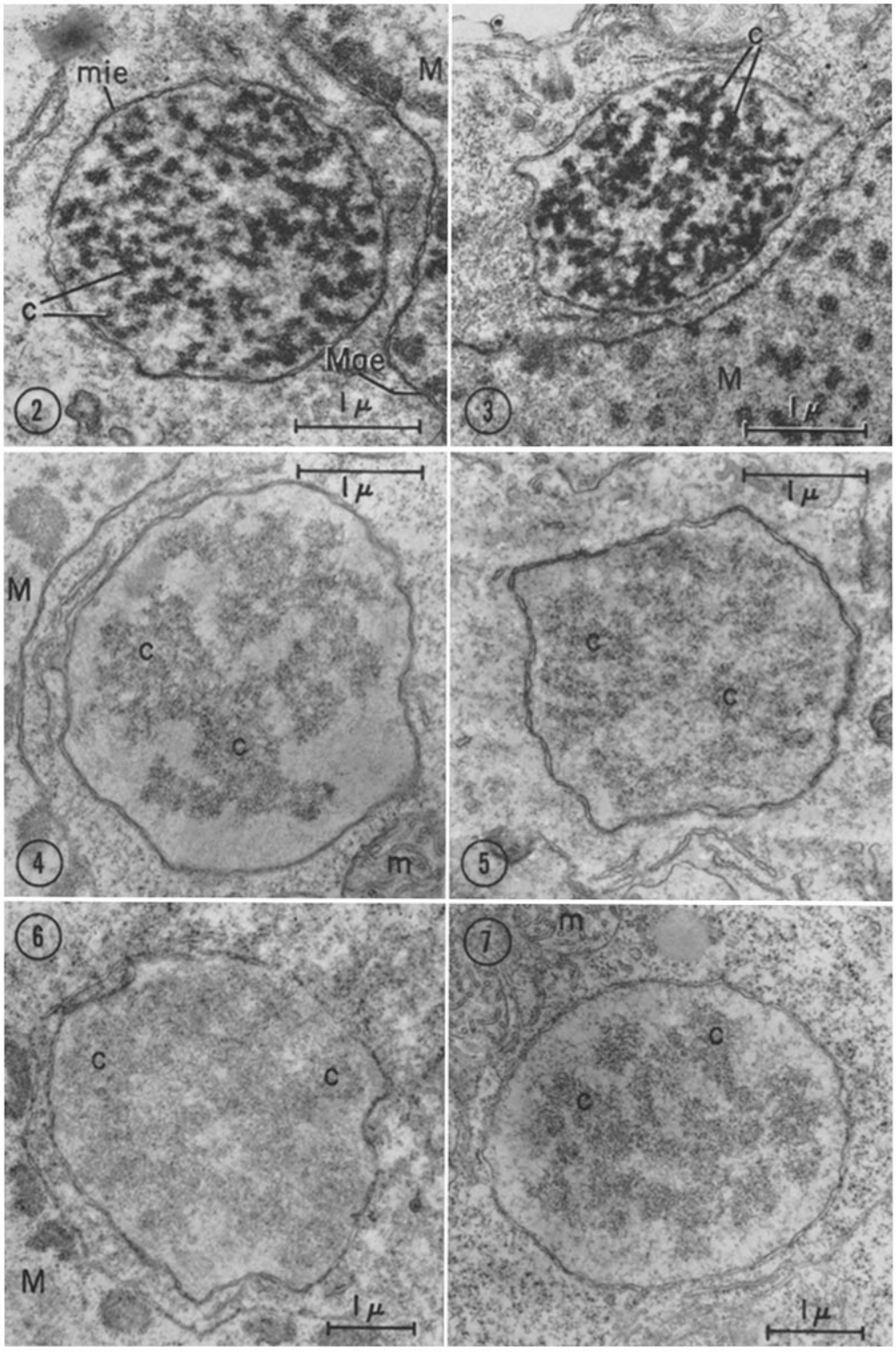
FIGURE 3 Micronucleus at 60 minutes (27 per cent completion of the cell cycle). × 19,000.

FIGURE 4 Micronucleus at 90 minutes (40 per cent completion of the cell cycle). × 19,000.

FIGURE 5 Micronucleus at 120 minutes (53 per cent completion of the cell cycle). × 19,000.

FIGURE 6 Micronucleus at 150 minutes (67 per cent completion of the cell cycle). × 13,000.

FIGURE 7 Micronucleus at 180 minutes (80 per cent completion of the cell cycle). × 15,000.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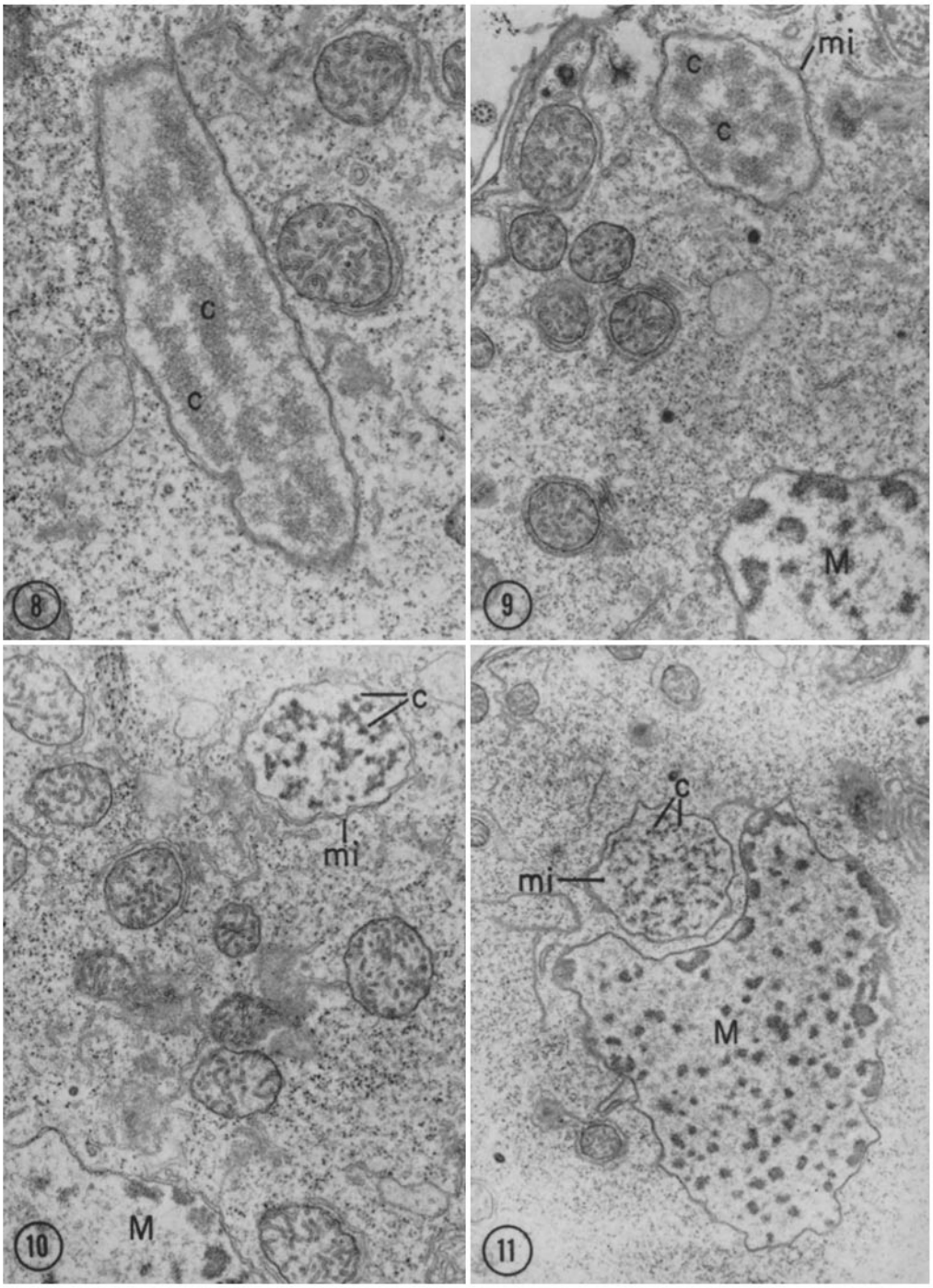


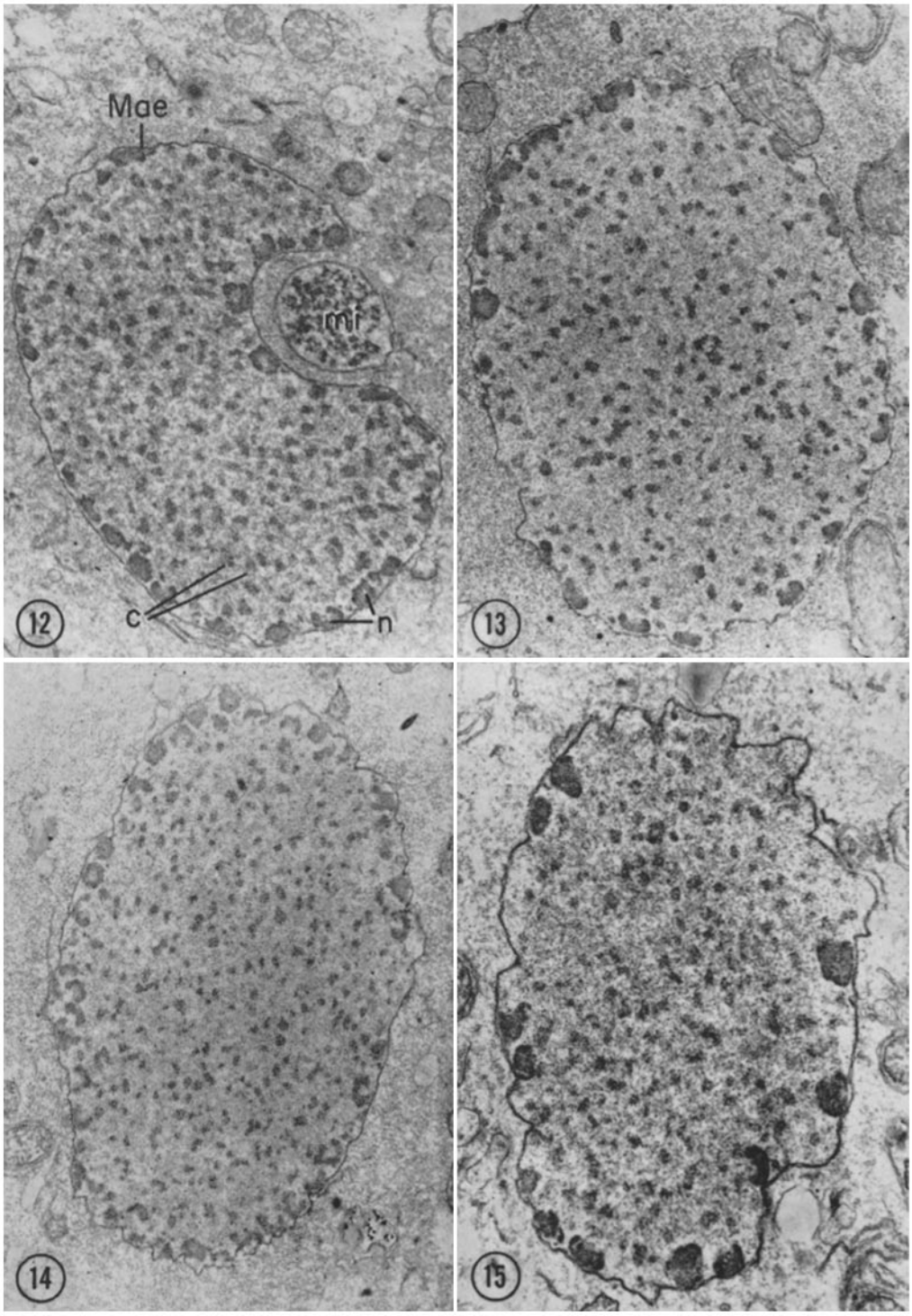
FIGURES 8 to 11 The appearances of the micronucleus at time 0; *i.e.*, cells selected in cytokinesis, 50 per cent of which have completed fission.

FIGURE 8 Elongated micronucleus with discrete clumps of chromatin (? chromosomes). × 9,000.

FIGURE 9 The micronucleus is seen at the periphery of the cell. The pattern of chromatin is very similar to that seen in Fig. 8. × 6,400.

FIGURE 10 The micronucleus is at the periphery of the cell and displays many fine clumps of dense chromatin. × 5,400.

FIGURE 11 The micronucleus lies in a depression in the macronucleus. It contains a large number of dense clumps of chromatin, characteristic of the early part of the cell cycle. × 5200.



FIGURES 12 to 18 The macronucleus at 30-minute intervals throughout the cell cycle. No striking changes are visible except for a decrease in density of chromatin bodies at 150 minutes (Fig. 16). No "RNA bodies" or nuclear "blebs" are seen.

FIGURE 12 Macronucleus and micronucleus at 30 minutes (13 per cent completion of cell cycle). × 3,000.

FIGURE 13 Macronucleus at 60 minutes (27 per cent completion of cell cycle). × 6,200.

FIGURE 14 Macronucleus at 90 minutes (40 per cent completion of cell cycle). × 4,500.

FIGURE 15 Macronucleus at 120 minutes (53 per cent completion of cell cycle). × 8,000.

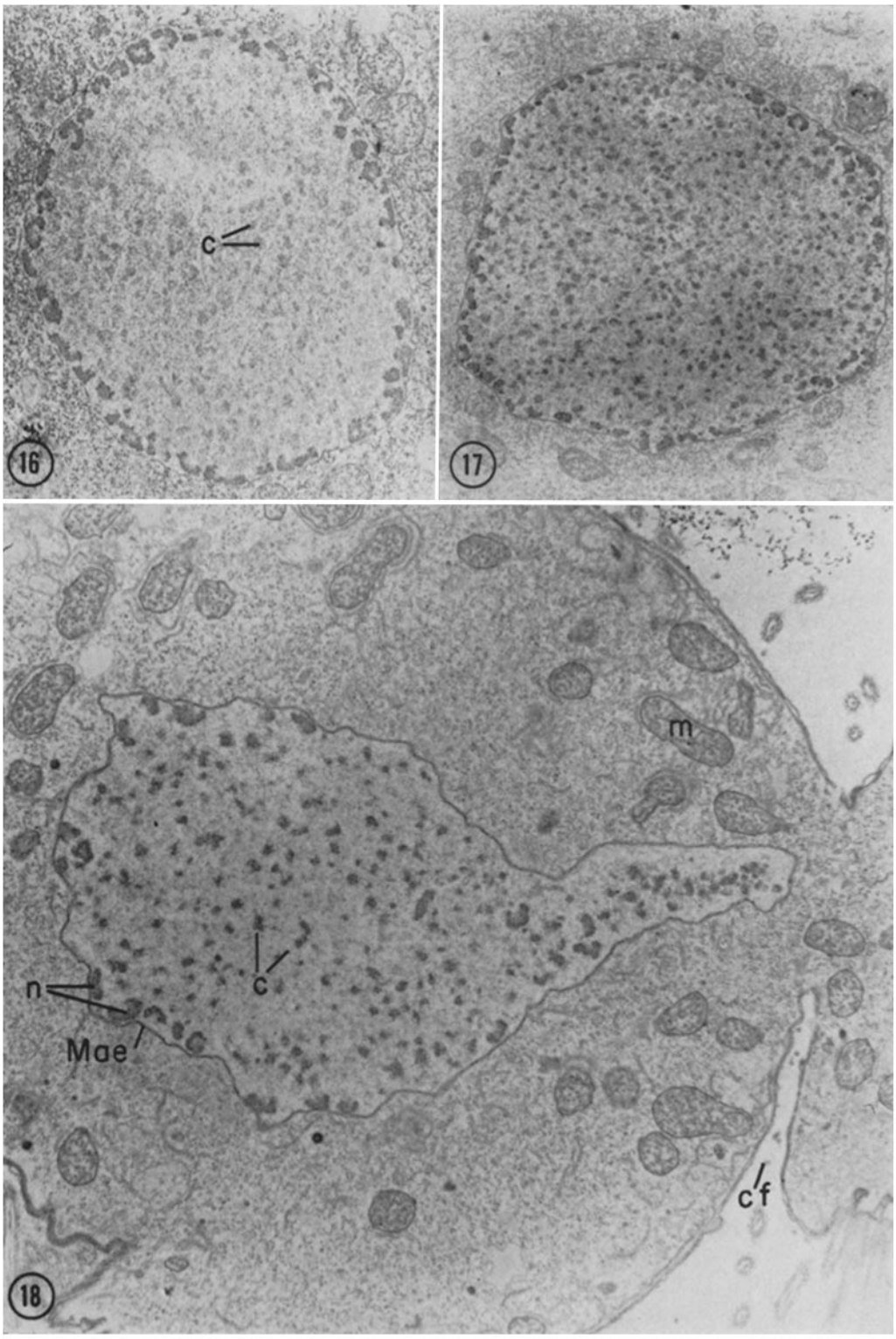


FIGURE 16 Macronucleus at 150 minutes (67 per cent completion of cell cycle). × 3,800.
FIGURE 17 Macronucleus at 180 minutes (80 per cent completion of cell cycle). × 2,600.
FIGURE 18 Macronucleus at time 0. The cell is in cytokinesis and the macronucleus of one daughter cell is elongated. × 2,000.

observed at any point in the cycle. It was not possible to demonstrate consistent changes in distribution or number of chromatin bodies or nucleoli during the cell cycle.[1]

At the time of cytokinesis, an elongated macronucleus apparently in the process of division is noted. It is possible that there is some increase in the density of chromatin bodies at this time (Fig. 18).

DISCUSSION

The results demonstrate that the micronucleus does undergo morphological changes throughout interphase. Furthermore, it is possible to determine, by inspection of the micronucleus, approximately in which one-fourth of the cell cycle a given organism is.

The hope of correlating observed structural variations with known biochemical events was not realized. The period of macronuclear DNA synthesis in 90 per cent of *Tetrahymena pyriformis*, strain HSM, under these conditions, begins at 27 to 30 per cent completion of the cell cycle (62 to 70 minutes) and continues until 60 to 67 per cent completion of the cell cycle (135 to 150 minutes) (15). Macronuclei fixed during pre-DNA synthesis (G_1), during DNA synthesis (S), and in the post-DNA synthesis period (G_2), however, have quite similar appearances.

The period of micronuclear DNA synthesis in strain HSM has been shown to be immediately after its telophase (16); *i.e.* while the cell is in cytokinesis, the macronucleus is dividing, and before the newly divided micronucleus has returned to the depression in the macronucleus. The micronucleus in Fig. 9 is similar to the dividing form seen in Fig. 8. Therefore, it is probably a late telophase nucleus or an elongated form sectioned transversely. The micronucleus shown in Fig. 10 probably follows that in Fig. 9 rather closely, since it is still separated from the macronucleus. Although one cannot state definitely that the micronucleus shown in Fig. 9 is not synthesizing DNA, its similarity to the division form which could not be in the S period (Fig. 8) argues against its being engaged in synthesizing DNA. The micronucleus in Fig. 10 seems more likely to be engaged in DNA synthesis since it is still separated from the macronucleus and appears to have progressed somewhat further from the time of division. The morphology of the micronucleus in Fig. 10, however, is similar to that of micronuclei in the early G_2 period (30 minutes). Breakdown or reformation of the nuclear envelope during division was not observed. Spindle formation was not seen in the present study and was not reported by Elliott (23). The existence of a spindle cannot be ruled out, however, since both studies utilized OsO_4 fixation near neutral pH, and it is possible that a better fixative for this structure, such as glutaraldehyde (24), or OsO_4 at acid pH with or without the addition of divalent cations (25), might demonstrate a spindle. It should be noted that since the micronuclear S period follows micronuclear division immediately, the G_1 period is non-existent or very short, and the majority of the micronuclear changes demonstrated (Figs. 2 to 7) take place during the G_2 period.

The present study, therefore, does not allow definition of any distinguishing morphological characteristics in either nucleus during the period of DNA synthesis. Perhaps only a small portion of the DNA is replicating at any given time.

The significance of the observed micronuclear changes is unknown. Since the G_2 period is very long in this nucleus, it might be thought that the changes represent early manifestations of prophase, not recognizable with the light microscope. The changes in micronuclear chromatin throughout the G_2 period, however, appear to consist of a decrease in number and in density of chromatin bodies, up to 180 minutes. At this time, when prophase is first recognizable with the light microscope, there is a reversal of this trend, with an increase in density of chromatin and its visualization as discrete bodies (? chromosomes). It seems more likely, therefore, that only the changes observed beginning at 180 minutes represent ultrastructural manifestations of prophase.

The significance of the earlier changes in micronuclear chromatin remains obscure, but perhaps these changes imply differences in chromosome coiling and/or functional state. Although the micronucleus undergoes meiosis and migration at the time of conjugation (26), specific micronuclear functions have not been demonstrated during vegetative growth and reproduction. In particular, RNA synthesis has been searched for

[1] When differences in magnification are considered, the apparent increase in number of chromatin bodies and nucleoli in Figs. 16 and 17 is not real. The number per section area remains approximately the same.

but not detected (D. M. Prescott, personal communication). Wells (27) has shown, however, that the production of viable amicronucleate strains from micronucleate strains by radiation is rare, and that those amicronucleates obtained have a longer generation time than the cells from which they were derived. Conversely, attempts to introduce micronuclei into amicronucleate cells by conjugation are infrequently successful. As Wells points out, apparently some change must take place in the cell to permit the loss or gain of a micronucleus. The implication of her results is that the micronucleus is performing some function during vegetative growth and reproduction, although the exact nature of its contribution remains elusive. Perhaps the difficulty in demonstrating specific micronuclear functions is due to the possibility that the micronucleus is active during only a small part of the cell cycle. Furthermore, its activity may be of a restricted nature, such as the synthesis of one or a few specific messengers. This hypothesis of change in micronuclear activity throughout the cell cycle is at least consistent with the observed changes in micronuclear morphology. It is hoped that future studies of segments of the cell cycle will elucidate precise functional correlates of these morphologic observations.

Scherbaum *et al.* (28) have demonstrated that heat-synchronized cells show some characteristics atypical of logarithmically growing cells; for example, the average cell volume increases up to 3 times that of log growth cultures, the DNA more than doubles in some cells, and the ratio of DNA/nuclear volume decreases during and immediately following the heat-shock treatment. The absence of "RNA bodies" and nuclear "blebs" budding off into the cytoplasm in over two hundred micrographs of logarithmically growing cells suggests that these structures reported by Elliott *et al.* in heat-shocked *Tetrahymena* may be products of the heat treatment. These authors, however, state that on occasion they have noted these structures in unsynchronized log phase cultures. Perhaps these discrepancies are due to differences between strains WH_6 and HSM. In strain HSM, at least, it appears more likely that transfer of nuclear material to the cytoplasm takes place at the molecular level, rather than by the more gross means of budding off of nuclear "blebs" into the cytoplasm.

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