STUDIES ON NUCLEAR STRUCTURE AND FUNCTION IN *TETRAHYMENA PYRIFORMIS*

I. RNA Synthesis in Macro- and Micronuclei

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ABSTRACT

Tetrahymena in the log phase of growth were pulse labeled with uridine-^{3}H, fixed in acetic-alcohol, extracted with DNase, and embedded in Epon. 0.5-μ sections were cut, coated with Kodak NTB-2 emulsion, and developed after suitable exposures. Grains were counted above macronuclei, above 1000 micronuclei, and above 1000 micronucleus-sized "blanks" which were situated next to micronuclei in the visual field by means of a camera lucida. An analysis of grain counts showed that micronuclei were less than $\frac{1}{2000}$ as active as macronuclei on the basis of grains per nucleus. Since micronuclei contained, on the average, about $\frac{1}{20}$ as much DNA as macronuclei, micronuclear DNA had less than 1% of the specific activity of macronuclear DNA in RNA synthesis. However, even this small amount of apparent incorporation was not significantly different from zero. Comparisons of the frequency distributions of labeled micronuclei with those of micronuclear "blanks" showed no evidence of a small population of labeled nuclei such as might be expected if micronuclei synthesized RNA for only a brief portion of the cell cycle. We conclude from these studies that there is no detectable RNA synthesis in *Tetrahymena* micronuclei during vegetative growth and reproduction.

INTRODUCTION

We have previously reported (Gorovsky, 1965, 1968) that micronuclei isolated from *Tetrahymena pyriformis* do not contain any RNA[1] which is detectable by light or electron microscopic techniques. Isolated macronuclei, on the other hand, do contain large amounts of RNA. Prior to carrying out studies on the histones of these isolated macro- and micronuclei (Gorovsky, 1968; Gorovsky and Woodard, 1968), we felt that it was necessary to corroborate these cytochemical findings by determining whether micronuclei *in situ* synthesize detectable amounts of RNA. To this end, we have carried out a quantitative radioautographic study of RNA synthesis in macro- and micronuclei by pulse-labeling *Tetrahymena* in the log phase of growth. Similar studies have been briefly reported by Alfert and Das (1959). The results obtained here are compared with recent radioautographic studies of RNA synthesis in micronuclei of *Paramecium caudatum* (Rao and Prescott, 1967) and *Paramecium aurelia* (Pasternak, 1967).

[1] Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; EDTA, ethylene diaminotetraacetic acid; DNase, deoxyribonuclease; RNase, ribonuclease; TCA, trichloroacetic acid.

MATERIALS AND METHODS

Culture Methods

Cells of mating type I, variety I of *Tetrahymena pyriformis* were grown axenically in an enriched proteose peptone medium (Dr. Frank Child, personal communication) containing 2.0% proteose peptone, 0.2% glucose, 0.1% yeast extract, and 0.003% sequestrine.[2]

Labeling and Radioautographic Procedures

20 ml of a log-phase culture of cells were incubated with 200 μc of tritium-labeled uridine (uridine-5-^{3}H, 2.0 c/mmole), for 3 min at room temperature. The cells were concentrated by centrifugation at 1000 *g* for 3 min, plus 2 min for deceleration. The supernatant was drawn off by aspiration and the cells were fixed in thin pellets with acetic acid–ethanol (1:3) for 1.5 hr at room temperature. The fixed cells were then rinsed with 100% ethanol, hydrated through a graded series of ethanols, and fragments of the pellets were treated with either DNase, RNase, or 0.003 M $MgSO_4$ for 2 hr at room temperature (Swift et al., 1964) followed by water, and 5% (w/v) TCA, 5°C, for 15 min. The pellets were then dehydrated and embedded in Epon (Luft, 1961). Sections were cut at a nominal thickness of 0.5 μ and mounted on gelatin-subbed slides (Caro, 1964). The DNase-treated sections were stained for 30 min in 1% (w/v) fast green FCF in 7% (v/v) acetic acid to make the micronuclei visible. Slides were then dipped in Kodak NTB 2 liquid emulsion, dried, and stored at 5°C over Drierite (Prescott, 1964). Development was carried out in Kodak D-19 developer for 2 min at 16°C after exposure times of 3 days and 2 wk.

Grain-Counting Procedures

The diameters of micronuclei which were cut in cross-section with no detectable material above or below them were measured with a filar ocular micrometer, and the average value was used for computing micronuclear area and volume, assuming a spherical shape for the micronucleus. Cytoplasmic and macronuclear areas were determined by carefully drawing the image of each measured cell and macronucleus with the aid of a Wild binocular camera lucida and then cutting out and weighing the drawings. Paper weights were calibrated by measuring 10 sheets of paper and weighing them and by cutting and weighing small measured circles from 10 sheets. A value of 0.61 g per cm^2 was obtained by both methods and was used for determining actual areas. Macronuclear volume was computed from the average macronuclear area by assuming that macronuclei were spherical.

Grain counts were made with a Wild phase-contrast microscope and a Wratten No. 25 filter for enhancing contrast. In the slides exposed for 3 days, grains above 1000 micronuclear cross-sections were counted. Grains above these micronuclei were classified either as peripheral (touching the edge of the nucleus) or as internal (clearly separated from the edge of the nucleus). Micronuclei were further classified according to their positions adjacent to, or removed from macronuclei. Grains were also counted over the macronucleus and cytoplasm of those cells in which a micronucleus was scored and in which the outlines of these compartments were clear enough to permit drawing. No other selection of macronuclei for counting was performed. Background grain counts for the macronucleus and the cytoplasm were considered to be the average grain densities within an ocular grid (area 3, 249 μ^2) measured in five different cell-free areas over each section, and were found to be 0.9–1.2 grains per 100 μ^2 for the four slides which were counted. In many cells, micronuclei were located so close to macronuclei that the possibility existed that many of the grains found above micronuclei actually resulted from β-particles which had their origin in the heavily labeled peripheries of the macronuclei. It was necessary, therefore, to devise other methods to determine background grain density for micronuclei: a circle was drawn with a draftsman's compass and carefully measured[3] so as to produce an image the size of an "average" micronuclear cross-section when viewed at the image plane with the camera lucida. This "micronuclear blank" was positioned in the plane of the section directly above the image of the micronuclear cross-section and was moved clockwise tangent to the micronucleus until it was in a position relative to the macronucleus which was (visually) judged to be the same as that of the actual micronucleus (Fig. 1). In sections in which no macronucleus was visible, the blank was positioned immediately above the micronuclear cross-section. Grains within the blank were counted and classified as peripheral or internal. Endogenous micronuclear incorporation was considered to be the difference between the sum of the grains over micronuclear cross-sections minus the sum of grains similarly located in the "micronuclear blanks."

In the slides exposed for 14 days, grain counts were made on 405 micronuclear cross-sections (and 405 "blanks"). In this case, however, only internal grains were counted since the higher grain densities and the resulting overlapping of silver grains made it impossible to determine peripheral grains accurately.

FEULGEN PHOTOMETRY OF NUCLEI IN SITU: Log-phase cells were pelleted by brief centrif-

[2] An iron-EDTA complex, available from Geigy Chemical Corporation, Ardsley, New York.

[3] Using a measuring magnifier, X7 magnification, 0.1-mm scale divisions, purchased from Bausch and Lomb, Rochester, New York.

ugation in a table-top clinical centrifuge. Drops of loosely packed cells were placed on gelatin-subbed slides, covered with a coverslip, and frozen on dry ice. Coverslips were removed, and the cells were post-fixed in 100% ethanol and stored in 100% ethanol (5°C) until used. This method of preparation resulted in large areas of well fixed, flattened cells. Slides were stained for DNA by the Feulgen reaction, dehydrated as usual, and mounted in refractive index oil for photometry. A random population of interphase cells plus all cells with micronuclei in metaphase or telophase was selected for measurement of macronuclear and micronuclear DNA amounts by the two wavelength method of Ornstein (1952) and Patau (1952).

FEULGEN PHOTOMETRY OF ISOLATED NUCLEI: Isolated nuclei (Gorovsky, 1965, 1968) were air dried on gelatin-subbed slides, fixed in 100% ethanol, and stained by the TCA-Feulgen method (Bloch and Godman, 1955). DNA amounts were measured by two wavelength photometry (Ornstein, 1952; Patau, 1952).

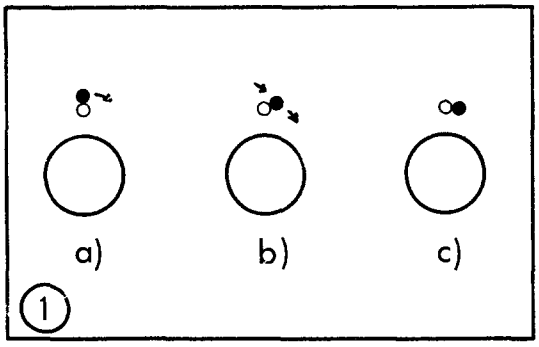

FIGURE 1 Diagram of method for measuring background labeling of micronuclei. The blank (closed circle) was placed above the micronucleus (small open circle) as in *a*). Blank was rotated clockwise about the micronucleus (*b*) until it was in a position, relative to the macronucleus (large circle), judged to be similar to that of the micronucleus (*c*).

RESULTS

The ratios of uridine incorporation between macro- and micronuclei were determined on four slides which were DNase extracted and fast green stained. Grain densities for macronuclei, cytoplasm, and background are presented in Table I. The similarities in grain densities, particularly of the heavily labeled macronuclei, showed that conditions which might contribute to variability between slides (such as differences in section thickness) were insignificant, and grain counts from the four slides were combined. It is interesting to note that the cytoplasm showed detectable incorporation even after the short pulse ($\leq$ 8 min) used to label the cells. In Table II, the grain densities of macro- and micronuclei have been computed, assuming that every nucleus which was counted was spherical and was cut through the center. It is clear from Table II that an average macronucleus was at least 2000 times more active in incorporation of tritiated uridine into DNase-resistant, TCA-precipitable material than a micronucleus. This incorporation was removable by RNase, and therefore grains were considered to be in RNA. Fig. 2 *a* shows a micronucleus next to a heavily labeled macronucleus.

The 2000-fold difference in incorporation between macro- and micronuclei is not a true representation of the differences in their ability to carry out RNA synthesis, because these nuclei also contain greatly different amounts of DNA. For determining the actual difference in the abilities of macronuclear and micronuclear DNA's to carry on RNA synthesis, the ratio between macro- and micronuclear DNA amounts in a log-phase population were measured by microspectrophotometry of Feulgen's-stained nuclei. On the average (38 measurements, nine on isolated nuclei, 29 on whole cells), macronuclei had 18.6 times more

TABLE I

Grain Densities of Radioautographs of Uridine-^{3}H-Labeled Tetrahymena

Slide No.	Background	Macronuclear grain density*	Cytoplasmic grain density*
1	0.012 grains/μ^2	0.289 grains/μ^2	0.009 grains/μ^2
2	0.009 grains/μ^2	0.232 grains/μ^2	0.007 grains/μ^2
3	0.009 grains/μ^2	0.276 grains/μ^2	0.007 grains/μ^2
4	0.009 grains/μ^2	0.264 grains/μ^2	0.005 grains/μ^2
Total (4 slides)	—	0.260 grains/μ^2	0.007 grains/μ^2

* Grain densities after subtraction of background.

TABLE II

Results of Radioautographic Studies of Uridine-^3H Incorporation into Macro- and Micronuclei of Tetrahymena

	Radius	Area	Volume	Total grains	Background grains	Net grains	Grains/unit volume	Grains/nucleus
Micronucleus	1.00 $\pm$0.03 μ(45)	3.12 μ^2	4.19 μ^3	702 (1000)	664 (1000)	38	0.030 grains/μ^3	0.126 grains
Macronucleus	4.57 μ	65.61 μ^2 (171)	399.69 μ^3	3,023 (171)	104	2,919	0.642 grains/μ^3	256.6 grains

Parentheses indicate the number of measurements which were made and serve to identify the parameters which were actually measured. All other values were derived from these.

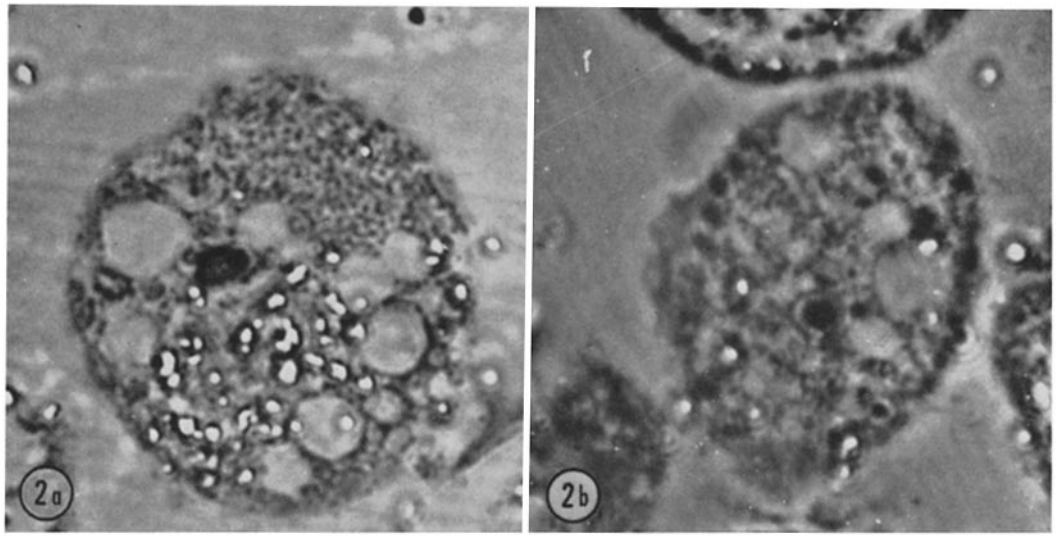

FIGURE 2 Radioautographs of uridine-^3H–labeled, DNase-extracted *Tetrahymena*. 0.5-μ sections were stained in fast green and exposed for 14 days. Photographs were taken with phase-contrast optics and a Wratten No. 29 red filter. (*a*) Micronucleus next to a labeled macronucleus. (*b*) Micronucleus with no adjacent macronucleus $\times$ 3500.

DNA than micronuclei. In terms of relative specific activity, then, macronuclear DNA was more than 100 times as active in RNA synthesis as micronuclear DNA (Table III). It should be pointed out, however, that the small difference observed between the total number of grains in micronuclei (702) and that in "micronuclear blanks" (664) was not significantly different from zero if the standard error of counting is considered to be the square root of the counts accumulated. We conclude from these data that in a population of log-phase *Tetrahymena*, DNA in micronuclei was less than $\frac{1}{100}$ as active as that of macronuclei in RNA synthesis and that the total number of grains above background in micronuclei was not significantly different from zero.

The above results refer to the "average micronucleus" in a large population of micronuclei. In order to determine whether some small proportion of micronuclei in the larger population was active (for example, if micronuclei synthesize RNA for a brief time in the cell cycle), the frequency distributions of labeled micronuclei were compared to those of "micronuclear blanks." By this means, it might be possible to detect a labeled population which was either too small or too lightly labeled (or both) to be detected when all of the micronuclei were considered together. Fig. 3 shows the frequency distribution of micronuclei and micronuclear blanks having one, two, three, and four or more total grains in the slides (3 days' exposure) used for determining the relative specific activities

TABLE III

Relative Specific Activities of Macro- and Micronuclear DNA in RNA Synthesis

	Relative DNA amounts	Grains/nucleus	Specific activity	Relative specific activity
Micronucleus	1.0 absorbance unit	0.126 grains	0.126 grains/unit DNA	1
Macronucleus	18.6 absorbance units	256.6 grains	13.8 grains/unit DNA	110

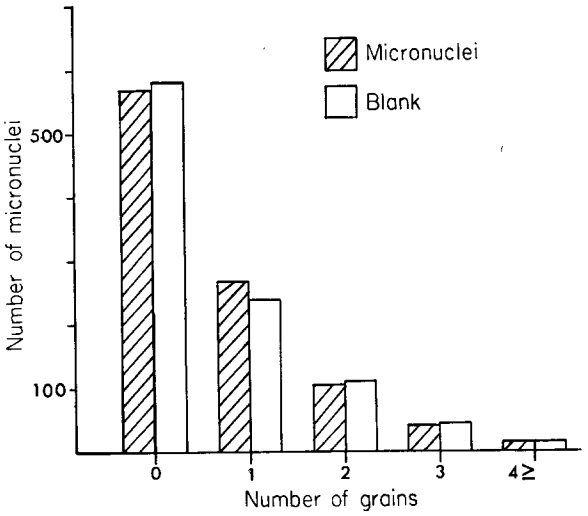

FIGURE 3 Frequency distribution of labeled micronuclei and micronuclear blanks. Total grains were counted in slides exposed for 3 days. There were no significant differences between micronuclei and blanks (Chi square: $0.5 > P > 0.3$).

of macro- and micronuclear DNA's. In slides exposed for longer times (14 days), it was possible to count only internal grains because the peripheral grains were so dense that they overlapped. No significant differences were detected in the frequency distributions in slides exposed for either 3 days (Fig. 3) or 14 days (Fig. 4). In summary, no evidence was found to indicate that micronuclei were active in RNA synthesis at any time in the cell cycle.

If micronuclei are inactive in RNA synthesis, then it is reasonable to assume that the grains which were observed above micronuclei (Table II) were due either to background or to beta particles which originated in macronuclei. As a result, micronuclei which were not adjacent to macronuclei (either because of fortuitous sectioning or because they had migrated away from their position in an inpocketing of the macronucleus) should show considerably less labeling than micronuclei which were near macronuclei (Fig. 2). In fact, micronuclei near macronuclei were labeled almost 12 times as intensely as those with no apparent macronuclei nearby (Table IV). The labeling (above background) observed in micronuclei with no adjacent macronucleus might have been due to thin macronuclear projections which surround micronuclei *in situ* but which were not visible by light microscopy.

DISCUSSION

RNA Synthesis in Vegetative Macro- and Micronuclei

The incorporation of uridine-^{3}H into acid-insoluble, DNase-resistant, RNase-digestible material strongly indicates that we are indeed measuring RNA synthesis. Labeling periods were restricted to short pulses so as to minimize the possibility of translocation of labeled material from one site to another, and we wish to emphasize the necessity for using short labeling periods to distinguish sites of synthesis of labeled molecules from sites of accumulation of these molecules. Nonetheless, even after the short labeling periods used here, cytoplasmic labeling has been observed in our studies. The ratio of cytoplasmic to macronuclear grain densities (Table I) is approximately 1 :37. Britten

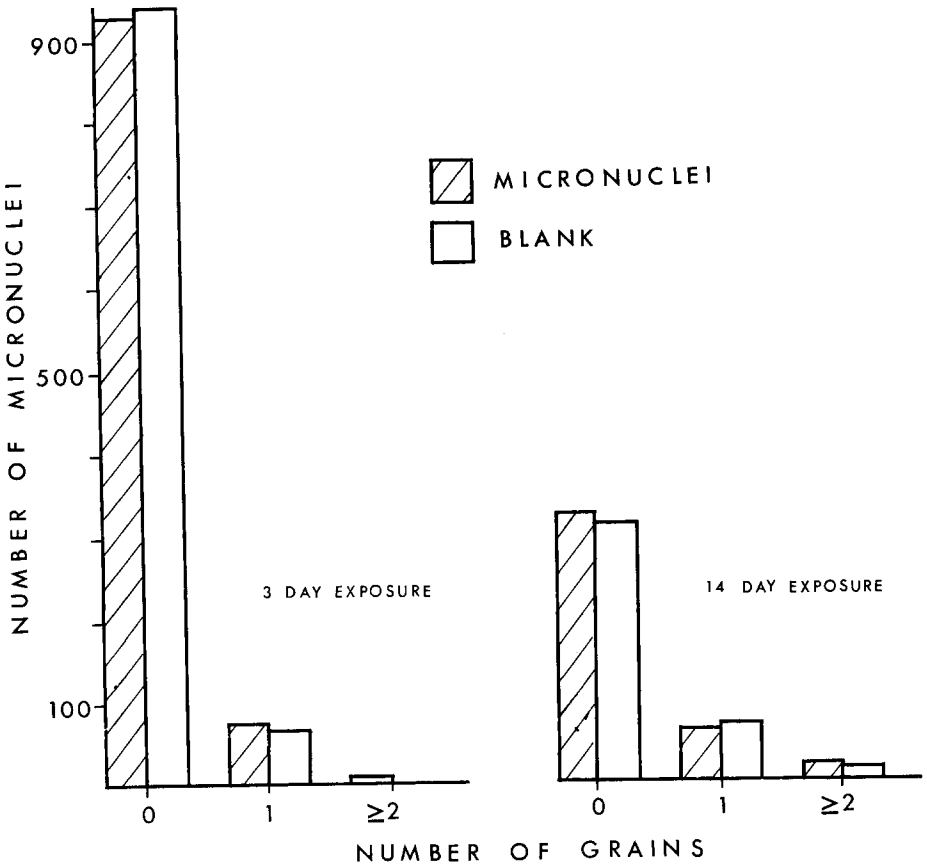

FIGURE 4 Frequency distributions of labeled micronuclei and micronuclear blanks. Internal grains only were counted in slides exposed for 3 days or 14 days. No significant differences were observed (Chi square; 3 days, $0.1 > P > 0.05$; 14 days, $0.8 > P > 0.7$).

TABLE IV

RNA Synthesis in Micronuclei with and Without Adjacent Labeled Macronuclei

	N	Grain density	Relative labeling intensity
Micronuclei adjacent to labeled macronuclei	798	0.692 grains/μ^3	12.1
Micronuclei with no adjacent macronuclei	202	0.057 grains/μ^3	1.0

(1959) has estimated that the macronuclear volume is only 3% of the cell volume in *Tetrahymena*. Therefore, total cytoplasmic labeling is about equal to macronuclear labeling after only 8 min of incorporation. It is impossible to determine by light microscope radioautographic methods whether this incorporation is due to independent cytoplasmic sites of RNA synthesis or to a rapid transfer to the cytoplasm of RNA's synthesized in the nucleus. Kumar (1968) has found newly synthesized RNA in the cytoplasm of *Tetrahymena* after 1 min labeling periods, and has characterized it by sucrose density gradient centrifugation as heterogeneously sedimenting and 4S RNA. He has also shown that detectable amounts of RNA with heterogeneous sedimentation properties are associated with cytoplasmic ribosomes after as little as 5 min of labeling, and newly formed stable ribo-

somal RNA can be detected in the cytoplasm after only 5.5 min. We feel, then, that this cytoplasmic incorporation probably represents newly synthesized messenger and soluble RNA's and perhaps even some ribosomal RNA's, which have been synthesized in the nucleus and rapidly transferred to the cy cplasm. A small percentage of the cytoplasmic labeling could, of course, represent synthesis of RNA by cytoplasmic organelles.

The validity of our conclusion on the absence of RNA synthesis in micronuclei depends on the methodology which we have employed to determine the background radioactivity in micronuclei. The radius of the average micronuclear section was determined by direct measurement, and the variability (standard error) in this measurement is lower than the standard errors for the number of grains counted over micronuclei or "micronuclear blanks." However, it is clear that a systematic error in determining the size of the circle to be used for the "micronuclear blank" would influence the results reported here. Therefore, the micronuclear blank was redrawn and carefully measured a number of times during this experiment, so as to insure that a single oversized blank was not used throughout. The good agreement between total labeling and the frequency distribution of labeled micronuclei and of "blanks" argue against any bias in size of the blank, since such a bias would necessarily have been of the exact size to produce identity between micronuclei and "blanks." Although we do not feel that the size of the "micronuclear blank" was overestimated (only overestimation influences our conclusions), the foregoing discussion clearly indicates that there is a finite uncertainty in this estimation.

In contrast to the situation above, a consistent error has been made in estimating the size of the macronucleus relative to that of the micronucleus. Since micronuclei are quite small, only sections which pass close to the center of a micronucleus will include enough of the nucleus to allow it to be clearly recognizable. Therefore, estimates of the diameter of micronuclei, though low, approximate the actual micronuclear diameter. On the other hand, macronuclei are much larger, and it is possible to recognize the macronucleus in sections which do not pass through the center of a macronucleus. Since we have not chosen the largest macronuclear cross-sections for measurement, it is clear that we have underestimated the macronuclear area (and hence, the volume) to a greater extent than the area of the micronucleus. Therefore, the ratio of 110:1 for the specific activity of macronuclear DNA relative to that of micronuclear DNA probably underestimates the actual disparity between them. In summary, although it is impossible to state that micronuclei are completely inactive in RNA synthesis in vegetative *Tetrahymena*, micronuclear DNA shows no detectable RNA synthesis under conditions in which macronuclear RNA synthesis is easily detected. Alfert and Das (1959) have reported similar results in abstract form.

Studies similar to these have been performed in a related ciliate, *Paramecium*. Rao and Prescott (1967) labeled synchronized *Paramecium caudatum* with tritiated uridine for 30 min, isolated nuclei by using Triton X-100 plus spermidine (Prescott et al., 1966), and made radioautographs of the isolated nuclei. They found that micronuclei labeled with approximately one-tenth the intensity of macronuclei. Labeled micronuclei were found only during the S period, which covered about 40% of the cell cycle, and the label was partially conserved for two subsequent divisions. It is difficult to interpret these results, for a number of reasons. First, 30 min is a relatively long labeling period, and it is certainly sufficient time for translocation of materials from their sites of synthesis. Second, in radioautographs of whole isolated nuclei, the nuclear membranes are apposed to the emulsion, and many nuclei, like those of *Tetrahymena* (Gorovsky, 1965, 1968), contain ribosome-studded nuclear envelopes. On the other hand, if the nuclear membranes have been removed by the action of the detergent during isolation, the possibility exists that labeled RNA from other cellular sites is adsorbed by the micronucleus during isolation. Until these possibilities are eliminated, it is difficult to be sure that the micronucleus in *P. caudatum* is actually a site of RNA synthesis.

Pasternak (1967) labeled synchronously dividing *Paramecium aurelia* for various lengths of time with tritiated uridine at different times in the cell cycle and studied the localization of RNA synthesis in thin sections by radioautography. He found that micronuclei showed a significant labeling through much of the cell cycle, with a distinct peak in the rate of incorporation occurring during the micronuclear S period. He also demonstrated that micronuclei showed almost twenty times the labeling intensity of the cytoplasm in amacronucleate cells (produced by unequal division of a mutant cell strain) which had been labeled for 60 min. Moreover, the micronuclei in the amacro-

nucleate cells showed only slightly less incorporation than micronuclei in normal controls labeled under identical conditions. These results strongly suggest that micronuclei in *P. aurelia* do synthesize RNA.

It should be noted that micronuclear RNA synthesis in *Paramecium* reportedly occurred largely during the micronuclear S period, which comprised about 40% of the cell cycle in *P. caudatum* and approximately 20% in *P. aurelia*. In *Tetrahymena*, micronuclear DNA synthesis occurs over approximately 10% of the cell cycle (see Flickinger, 1965; Woodard, Kaneshiro, and Gorovsky, unpublished observations), and it seems likely that if RNA synthesis occurred in such a large fraction of the cells in a log-phase population, this synthesis would have been detected in our analysis of the large number (1, 405) of micronuclear cross-sections. Cytological, cytochemical, genetic, and biological studies which bear on the problem of RNA synthesis and genetic activity in *Tetrahymena* and other ciliates will be discussed below.

Genetic Activity in Micronuclei of Tetrahymena and Other Ciliates

A number of lines of evidence support the conclusion that micronuclei in *Tetrahymena* are genetically inactive, particularly during vegetative growth: (*1*) Micronuclear RNA synthesis was not detectable in intact log-phase cells by radioautographic techniques. (*2*) The morphological elements (nucleoli, heterogeneous granules) in which the nuclear RNA's of most cells appear to be located were not found in the micronuclei of *Tetrahymena* (Gorovsky, 1965, 1968). (*3*) Amicronucleate individuals make up significant percentages of wild collections of *Tetrahymena* (Pacific collections, 65% amicronucleate; Central and South America, 60%; United States, 33%; Europe, 39%) made by Elliott and his coworkers (Elliott et al., 1964), and vigorous amicronucleate strains of *Tetrahymena* have been maintained in vegetative culture for extended periods of time (Corliss, 1954). Therefore, the micronucleus does not appear to perform any function essential for vegetative growth. (*4*) Sonneborn (1954) reported that a dominant gene in the micronucleus of heterocaryotic *Paramecium* does not influence the phenotype of the cell, and Pasternak (1967) showed that dominant genes in the micronucleus were incapable of maintaining the presence of mate-killer particles in *Paramecium* under conditions in which it has been estimated that only one gene product per cell is sufficient to do so (Gibson and Beale, 1962). Therefore, although it appears that micronuclei of *Paramecium* are capable of RNA synthesis (Rao and Prescott, 1967; Pasternak, 1967), the genes which have so far been introduced into micronuclei have no detectable function.

In opposition to the evidence indicating that the micronucleus in *Tetrahymena* is inactive during vegetative growth are the studies of Wells (1961). She irradiated *Tetrahymena* and found that the frequency of recovery of viable amicronucleate clones was low, despite the presence of numerous amicronucleate cells after irradiation. She also found that an amicronucleate clone grew more slowly than its parent micronucleate clone, and concluded that "the ciliate micronucleus unquestionably contributes information to the cell during asexual growth and reproduction." However, we feel that her results are subject to a different interpretation. The failure of many amicronucleate cells produced by irradiation to give rise to viable clones may be due to the fact that cells selected by this criterion are necessarily damaged by radiation, while cells with micronuclei may include cells which have escaped serious radiation damage. Moreover, since replication of the micronucleus must normally be integrated into many of the metabolic pathways of the cell (such as those involved in nucleic acid and membrane biosynthesis, for example), some imbalance in any of these pathways may be caused by micronucleus removal and could explain the reduced viability and vigor of amicronucleate cells which Wells observed. The two most striking observations made by Wells—that viable amicronucleate clones can be recovered after X-irradiation of micronucleate cells and that reintroduction of micronuclei into these clones is rarely successful and does not restore the properties of the original micronucleate parents—indicate that the micronucleus does not contribute genetic information essential to vegetative growth.

Although it is tempting to generalize our findings on micronuclear function in *Tetrahymena* to other ciliates, the foregoing discussion indicates that differences may exist even in related ciliates such as *Tetrahymena* and *Paramecium*. In *Tetrahymena*, micronuclei appear to be genetically inactive and are frequently lost by cells in nature. In *Paramecium*, the micronucleus appears to synthesize some RNA, although its genetic importance has yet to be demonstrated. However, *Paramecium* is rarely, if ever, collected in nature in the amicro-

nucleate condition, and amicronucleate strains have been reported in the laboratory only infrequently (Wichterman, 1953). In *Euplotes*, a more distantly related ciliate, microsurgical removal of the micronucleus invariable results in death, although removal of cytoplasm by similar techniques has little effect (Taylor and Farber, 1924). Moreover, amicronucleate *Euplotes* have not been reported. Perhaps it is not surprising that a group so diverse as the ciliates have evolved apparently different degrees of integration of micronuclei into the asexual functions of the cell, and it should be interesting to attempt to further correlate the "indispensability" of the micronucleus with its capacity for RNA synthesis in different species.

In conclusion, we suggest that the micronucleus in *Tetrahymena* is genetically inactive and does not synthesize or contain RNA during vegetative growth and reproduction. Additional cytochemical and radioautographic studies are necessary to extend this conclusion to micronuclei during conjugation, although preliminary experiments (Gorovsky, 1968; Gorovsky and Woodard, unpublished) have failed to detect any micronuclear RNA synthesis at that time. Clearly, isolated macro- and micronuclei of *Tetrahymena* should provide interesting material for studying the control of nuclear functions, since they seem to represent the same genetic information in extremely different states of activity.

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