THE DISTRIBUTION OF DNA AMONG DIVIDING MITOCHONDRIA OF *TETRAHYMENA PYRIFORMIS*

JOHN A. PARSONS and RONALD C. RUSTAD

From the Department of Biology, San Diego State College, San Diego, California 92115, and the Departments of Radiology and Biology, Western Reserve University, Cleveland, Ohio 44106

ABSTRACT

A squash technique was developed for log phase *Tetrahymena pyriformis* which permitted the resolution of over 100 individual mitochondria from a single cell. Mitochondria incorporated thymidine at all stages of the cell cycle, even when nuclear DNA synthesis was not occurring. During the stage of macronuclear DNA synthesis, however, there was a significant increase in the extent of mitochondrial labeling. Low radioautograph background suggests that mitochondrial DNA is synthesized at the mitochondria themselves. All mitochondria incorporated thymidine-^{3}H within one population-doubling time. Grain counts also showed that the amount of mitochondrial label was retained for four generations and that this label remained randomly distributed among all mitochondria during this time. The results are not consistent with any theory of de-novo or "microbody" origin of mitochondria, but do support the hypothesis that mitochondria are produced by the growth and division of preexisting mitochondria. The stability of the mitochondrial DNA and its distribution among daughter mitochondria satisfy two prerequisites for a genetic material. The possibility is discussed that some of the genetic information for the mitochondrion is contained in the DNA associated with this organelle.

INTRODUCTION

The mechanism of the origin of mitochondria has been a subject of controversy for many years. To date, experiments have provided no clear demonstration of how these organelles are formed during normal cell growth. Three major theories on the origin of mitochondria exist: de-novo formation from newly synthesized materials, differentiation of cytoplasmic "microbodies" from either newly synthesized or preformed cellular membranes, and division of preexisting mitochondria.

Thymidine-^{3}H is incorporated into a mitochondrial DNA which is conserved during four generations of logarithmic growth (14, 15). The mitochondrial localization and stability of this DNA has been confirmed by Stone and Miller (22). The present experiments describe the random distribution of mitochondrial DNA during four cell cycles and the incorporation of thymidine-^{3}H by all mitochondria during a single cycle of cell growth. The results are incompatible with any hypothesis of de-novo or "microbody" origin of mitochondria, but provide strong support for the hypothesis that mitochondria are formed by the growth and division of preexisting mitochondria.

MATERIALS AND METHODS

Culture Conditions

The micronucleate strains HSM and variety 6, mating type III (6, III) of *Tetrahymena pyriformis* were grown in a 1% proteose-peptone, 0.1% liver fraction "L" (stock) medium, as previously described

(15). All radioactive labeling was done in a chemically defined medium modified from Elliott and Hayes (3). In addition to the regular ingredients, the defined medium contained 400 mg/liter proteose-peptone and was prepared with half the pyrimidine content listed by Elliott and Hayes or with no pyrimidines added.

Radioactive Labeling and Cell Sampling

Thymidine-^{3}H (Schwarz BioResearch, Inc., Orangeburg, N. Y.) 3.0 and 6.0 c/mmole was used at a final concentration of 50–100 μc/ml medium. During short labeling experiments, 40 μg of streptomycin sulfate and 25 μg of sodium penicillin "G"/ml medium were used for prevention of bacterial contamination. Cells were removed from the isotope-containing medium with a capillary pipette after 15 min (HSM), 30 min (6, III), or one population-doubling time (both strains) and transferred to iced stock medium. Cell squashes were prepared immediately for radioautography.

In experiments with prelabeled cells, thymidine-^{3}H was added aseptically to freshly inoculated cultures which were then incubated for 1 wk. The cells were collected by a 2-min centrifugation at full speed in an International Clinical Centrifuge. Cells were resuspended and washed three times by centrifugation in distilled water. The cells were then suspended in the defined medium containing no pyrimidines. Streptomycin and penicillin were added at concentrations stated above, and the cells starved for 24 hr. During this period, all food vacuoles were eliminated and no population increase could be detected with the Coulter counter. The starved culture was centrifuged, and the cells were resuspended in the stock medium with streptomycin and penicillin. Some cells were squashed for radioautography at this stage (time zero), and other individual cells were isolated in capillary pipettes for sampling at later division stages. One daughter cell was squashed for radioautography approximately 1 hr after each division.

Squash Procedure

Mitochondria streaming from freshly squashed cells swell and rupture in less than 30 sec in the defined medium. The following squash procedure was devised which could retain, on glass slides, over 100 distinct mitochondria, 1–2 μ in diameter, from a single cell. An intact cell, covering an area of approximately 1300 μ^2, can thus be spread over at least 20,000 μ^2 of slide area.

Glass slides were cleaned in chromic acid cleaning

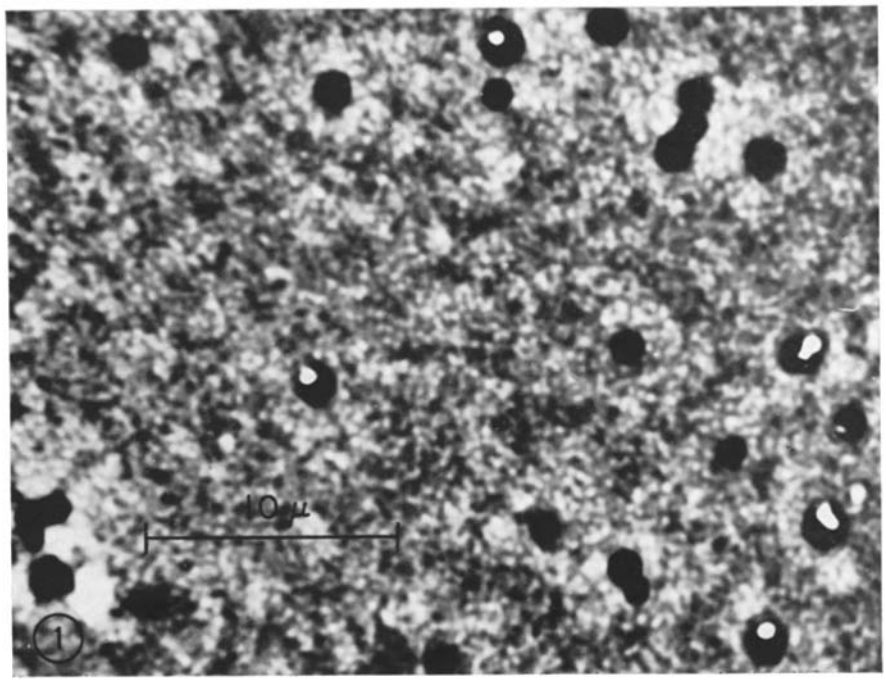

FIGURE 1 Phase-contrast photomicrograph of a squash radioautograph indicating the specificity of mitochondrial labeling. The *Tetrahymena* cell was grown in thymidine-^{3}H for 15 min. Large black circles are mitochondria, smaller white dots are the negative phase images of radioautograph grains. × 2,000.

solution, rinsed several hours in running tap water and two 5-min changes of distilled water, dried, and kept in sealed slide boxes until used. Two "squashing solutions" were effective in inhibiting mitochondrial swelling long enough for dispersal and fixation to occur. A solution of 1% proteose-peptone, 4.5% formalin worked most consistently, but a solution of 1% proteose-peptone, 9% formalin, 0.01 M KCl sometimes gave better results if cells were still in lag phase. The squash procedure worked most consistently with cells in log phase. Under a dissecting microscope, two drops of the "squashing solution," each less than 2 mm in diameter, were placed on a cleaned slide about 12 mm apart. A similar (same size) drop of stock medium containing the *Tetrahymena* to be squashed was placed between the first two droplets. A clean, number 1, 22-mm^2 cover slip was centered by hand over this area, the two opposite points of the cover slip protruding over the edges of the slide. The cover slip was lowered with the point of a metal-handled dissecting needle until it made contact with the three droplets. The amount of fluid in the three droplets was sufficient to just cover the area under the cover slip with a layer thin enough to rupture the cell membrane. The cover slip was gently tapped with the blunt end of the dissecting needle. The slide was then placed on the stage of a phase-contrast microscope, and the cell located under low power. The microscope condensor was adjusted for oil immersion phase contrast so that the cell appeared in darkfield on low-power magnification. Dispersing mitochondria were easily seen, and further spreading could be achieved with a few taps of the dissecting needle. The time between breaking of the cell membrane and gelation of the cell amounted to no longer than 30 sec. The mechanical stage coordinates were marked on the slide for the purpose of aiding the later location of the squashed cell. The slide was frozen on a block of dry ice, the cover slip was removed, and the slide air dried.

Procedures for identification of the mitochondria and for the preparation of radioautographs have been described previously (15).

Grain Counts

A drop of distilled water and a cover slip were placed on an radioautograph which then was examined by oil immersion phase-contrast microscopy. The distribution of radioautograph grains was determined for 100 mitochondria per cell. Only cells showing at least 100 individual mitochondria in radioautographs were counted. If the nucleus was fragmented, resulting in a high-grain background, the cell was not counted.

Statistical Analysis

The grain distribution data were plotted in the form of histograms. The Poisson distribution, describing the randomness of a small number of events in a large population, is given by the following formula:

$$P_g = \frac{(\bar{x})^g \cdot e^{-\bar{x}}}{g!}$$

where P_g is the probability that a given mitochondrion will have g grains, and $\bar{x}$ is the observed average number of grains per mitochondrion. The "goodness of fit" of experimental data can be tested by using the following form of this equation described by van Tubergen and Setlow (28) and Luck (6):

$$\ln(P_g \cdot g!) = g \ln \bar{x} - \bar{x}$$

A plot of ln $(P_g \cdot g!)$ versus g for a true Poisson distribution gives a straight line with slope = ln $\bar{x}$ and intercept = $-\bar{x}$ for $g = 0$.

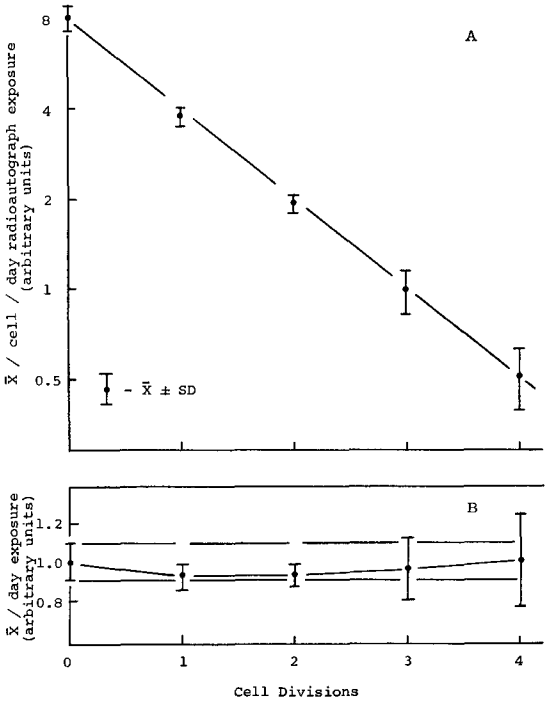

FIGURE 2 Semilogarithmic plot of the mitochondrial grain count versus division stage for thymidine-^{3}H-labeled strain 6, III during four growth cycles in unlabeled medium. The upper curve (*A*) shows the average mitochondrial grain count ($\bar{X}$) per cell. The solid line represents a halving of the grain density at each division stage. The lower curve (*B*) shows the total mitochondrial label at each division stage.

RESULTS

Stability of the Mitochondrial Label

Wherever possible, experiments were carried out with cells at the stage of logarithmic growth. The squash technique employed allowed a more than tenfold increase in resolution while retaining clearly visible mitochondria. As described previously, essentially all cytoplasmic thymidine-^{3}H labeling was associated with mitochondria (Fig. 1). Background grains not associated with mitochondria or intact nuclei amounted to less than one grain per 1000 μ^2 of slide area. The squash procedure occasionally caused rupture of the macronuclear membrane, resulting in a high background immediately around that cell. Cells which showed this nuclear fragmentation were never used for obtaining experimental data. The stability of the mitochondrial label is indicated by the data from one of four similar experiments. The average mitochondrial grain count of thymidine-^{3}H–labeled cells grown for four generations in unlabeled medium was presented in a previous paper (15). Since the average number of grains per mitochondrion did not differ significantly among individual cells within a single experiment, the data were grouped within each division class. Fig. 2 shows these data plotted as division class versus mitochondrial label density per cell and per total population of cells. It is clear that the average mitochondrial grain count per cell is halved each cell generation and that the total mitochondrial label is conserved for at least four generations.

Experiments with Prelabeled Cells

Individual cells, labeled with thymidine-^{3}H and incubated in pyrimidine-free medium, were isolated in capillary pipettes containing nonradioactive stock medium. The first division of these isolated cells occurred in approximately 6 hr; subsequent divisions occurred at 3- to 4-hr intervals. Squashes of one daughter cell were made approximately 1 hr after each cell division. Fig. 3 shows the grain distribution within the mitochondrial population for the data shown in Fig. 2. The bars represent the data; the dots represent the theoretical Poisson distribution for a population having the same average grain count ($\bar{x}$). For better comparsion of the distribution of radioautograph grains on mitochondria with the theoretical distribution, the data were plotted in Fig. 4 as ln ($P_g \cdot g!$) versus g, as suggested by van Tubergen and Setlow (28). The observed data (open circles) fall fairly close to the theoretical Poisson (solid line) distribution. However, at divisions one and two, counts of mitochondria with four grains were below the expected frequency. This is probably because of the lower contrast of mitochondria in

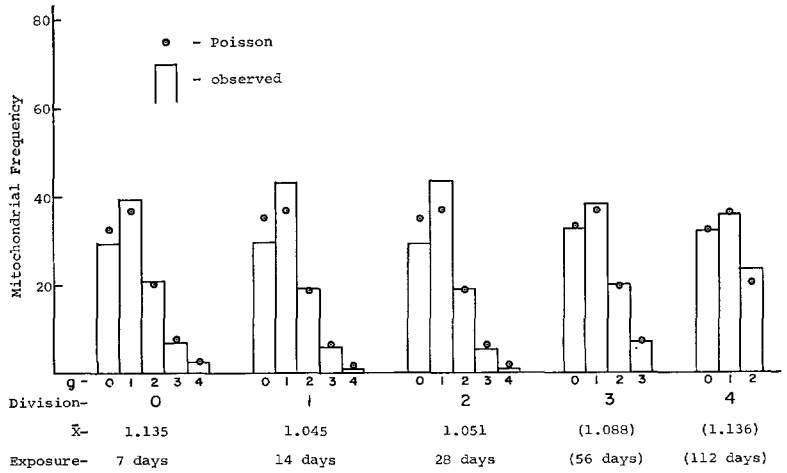

FIGURE 3 Histograms showing the distribution of grains on mitochondria during four division cycles in unlabeled medium. The data are from the experiment shown in Fig. 2. Theoretical Poisson distributions are calculated from the observed average mitochondrial grain counts ($\bar{X}$). Data in parentheses (divisions *3* and *4*) are calculated from shorter radioautograph exposure times. Grain count frequencies below 1 are omitted from the figure.

this particular experiment. In three other experiments with prelabeled cells, grain distributions very closely fit the theoretical Poisson distribution.

Short Pulse-Label Experiments

Cells were grown for 3 days in the defined medium. Population growth studies showed that these cells were still in the stage of logarithmic growth. Thymidine-^{3}H was added, and cell squashes were prepared after 15 min (strain HSM) or 30 min (strain 6, III) of incubation in the radioactive medium. Radioautographs of these squashes showed cells at one of three stages of nuclear DNA synthesis. Cells were found with a solid mass of

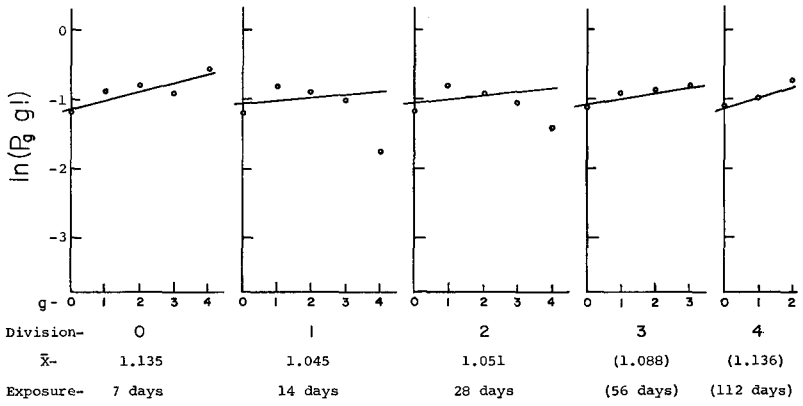

FIGURE 4 Ln($P_g \cdot g!$) versus g plots of the data from Fig. 3. Solid lines represent theoretical Poisson distributions for the observed average mitochondrial grain counts ($\bar{X}$).

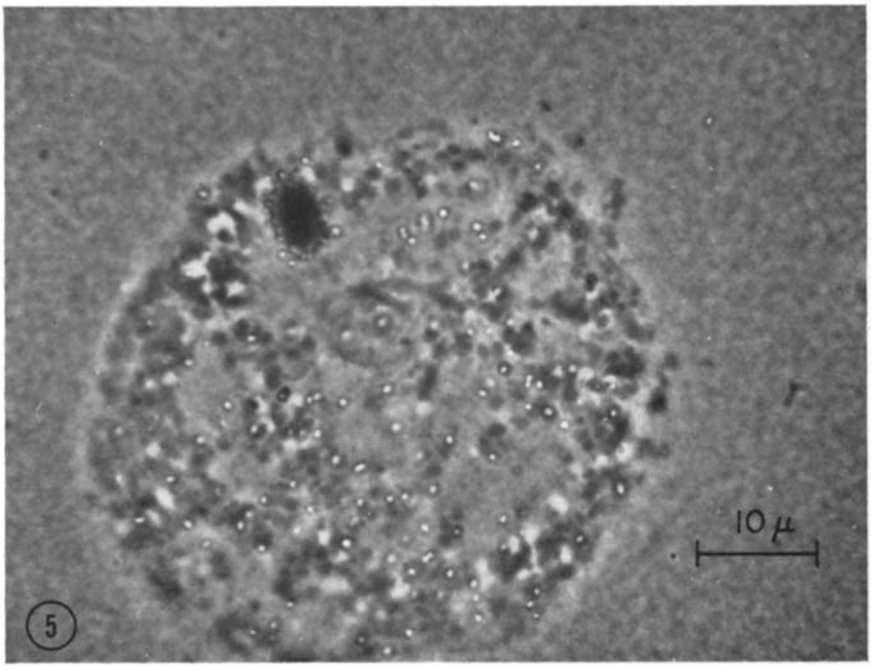

FIGURE 5 Phase-contrast photomicrograph of a radioautograph showing a partially squashed cell. The cell was pulse-labeled with thymidine-^{3}H at a time when micronuclear DNA synthesis was occurring. The micronucleus visible at the upper part of the cell is covered by a solid mass of silver grains. Single radioautograph grains show as white spots. × 1,000

grains over the micronucleus or over the macronucleus or with no more grains over either nucleus than over the cytoplasm (Figs. 5–7). In the latter case, grains over the nuclei were associated with mitochondria on the nuclear surface. Even when no nuclear labeling had occurred during the exposure to thymidine-^{3}H, a significant number of grains were found over the mitochondria. Of several thousand cells observed, all cells showed some labeled mitochondria. Since mitochondria were labeled at times when nuclear incorporation was not occurring, synthesis of the mitochondrial label could not have taken place in the nucleus. Because radioautograph background was very low and almost all cytoplasmic grains contacted mitochrondria, synthesis of mitochondrial DNA was probably occurring in or on the mitochondria themselves.

Table I summarizes the average mitochondrial grain counts of both strains of *Tetrahymena* after short pulse labeling with thymidine-^{3}H. The rate of mitochondrial labeling was essentially the same whether or not micronuclear DNA synthesis was occurring. The data show, however, that there is a significant increase in the rate of mitochondrial labeling when macronuclear DNA synthesis occurs.

The grain distribution within the mitochondrial population after short pulse labeling of both *Tetrahymena* strains is shown in the $\ln (P_g \cdot g!)$ versus g plots in Figs. 8 and 9. Although the 30-min labeling shown in Fig. 9 closely fits a Poisson distribution, Fig. 8 shows a grain distribution after 15 min of labeling (strain HSM) which is distinctly different from a Poisson distribution. This deviation is more marked in cells showing the higher rate of mitochondrial labeling during macronuclear DNA synthesis (solid circles). The data suggest that the mitochondrial population is not randomly labeled, but does, in fact, have most of the label located in a few mitochondria. The most plausible explanation for this is that synthesis of a large macromolecule or group of macromolecules in one mitochondrion is discontinuous, but that synthesis is occurring in some mitochondria at any time within the cell growth cycle.

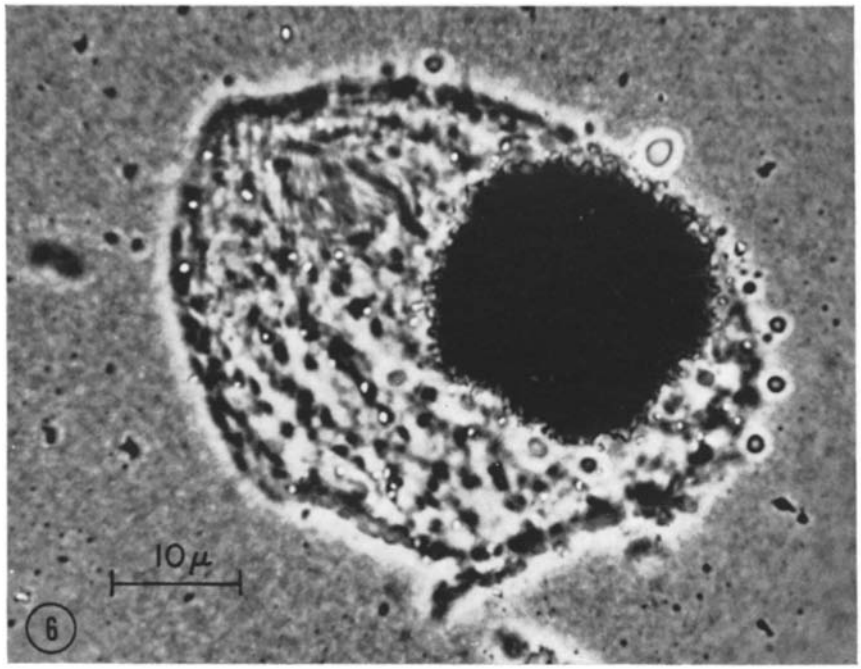

FIGURE 6 Phase-contrast photomicrograph of a radioautograph showing a cell pulse labeled with thymidine-^{3}H during the stage of macronuclear DNA synthesis. The macronucleus located at the upper right corner of the cell is covered by a solid mass of silver grains. × 1,000

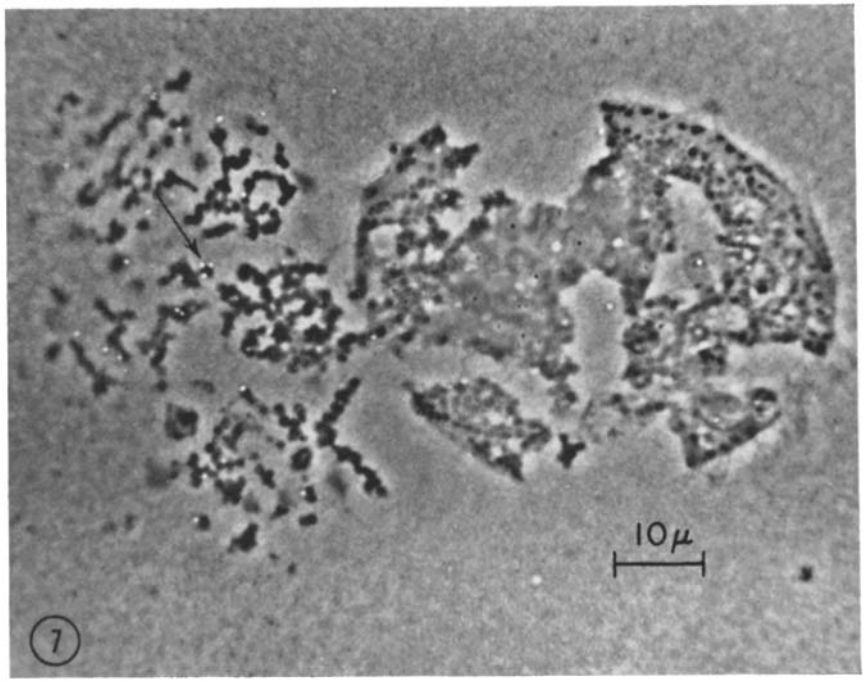

FIGURE 7 Phase-contrast photomicrograph showing a cell pulse-labeled with thymidine-^{3}H at a stage when nuclear DNA synthesis was not occurring. The macronucleus is visible as a cloudy area in the center of the cell body. Note radioautograph grains on mitochondria spread to the left of the main cell body; one mitochondrion (arrow) shows three contacting grains. ×1,000

TABLE I

Average Mitochondrial Grain Counts ± Standard Deviation of Tetrahymena after Pulse Labeling with Thymidine-^{3}H

Strain	Pulse duration	DNA synthesis stage	N*	$\bar{X} \pm$ SD
	(*min*)			
HSM	15	Micronuclear synthesis	6	0.128 ± 0.041
		Macronuclear synthesis	20	0.220 ± 0.044‡
		No nuclear synthesis	54	0.100 ± 0.031
6, III	30	Micronuclear synthesis	10	0.170 ± 0.080
		Macronuclear synthesis	28	0.265 ± 0.080‡
		No nuclear synthesis	43	0.148 ± 0.054

* Number of cells counted, one hundred mitochondria per cell.

‡ Statistically significant.

Labeling for One Population-Doubling Time

Log phase cultures of both strains of *Tetrahymena* were grown in chemically defined medium containing thymidine-^{3}H for a period equivalent to one population-doubling time. Cell squashes and radioautographs were prepared as before. A ln ($P_g \cdot g!$) versus g plot of the mitochondrial grain distribution is shown in Fig. 10. It is clear that this grain distribution again corresponds to a Poisson distribution. All mitochondria must, therefore, have incorporated thymidine-^{3}H during one population-doubling time.

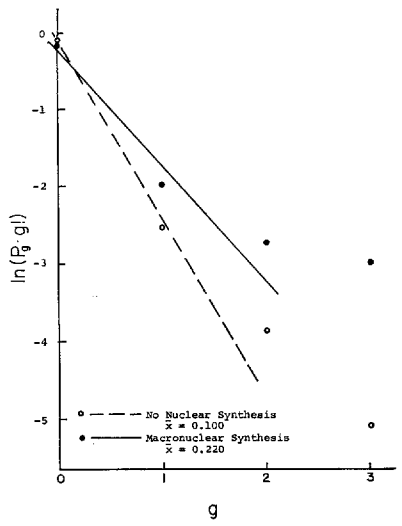

FIGURE 8 Ln($P_g \cdot g!$) versus g plots of the mitochondrial grain distribution in strain HSM after 15-min pulse-labels of thymidine-^{3}H. The lines represent theoretical Poisson distributions for the observed average mitochondrial grain counts ($\bar{X}$).

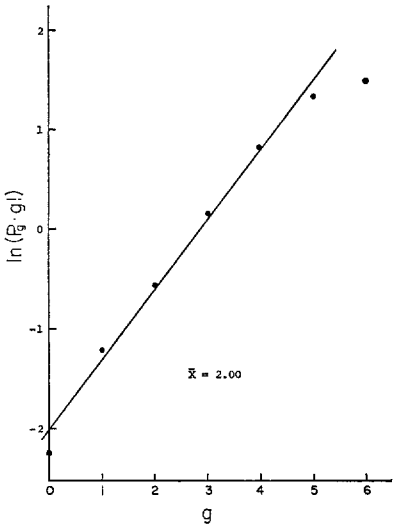

FIGURE 10 Ln($P_g \cdot g!$) versus g plot showing the distribution of grains on mitochondria in strain 6, III after labeling with thymidine-^{3}H for one population-doubling time. The solid line represents the theoretical Poisson distribution for the observed average mitochondrial grain count ($\bar{X}$).

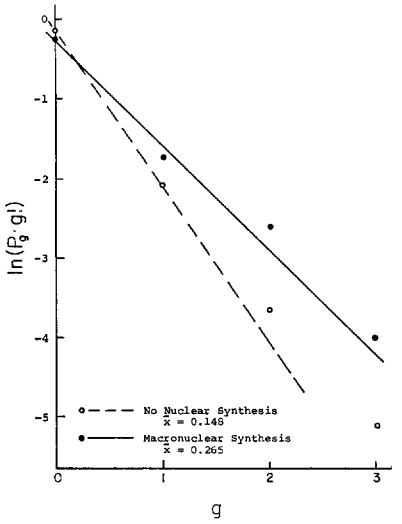

FIGURE 9 Ln($P_g \cdot g!$) versus g plots of the mitochondrial grain distribution in strain 6, III after 30-min-pulse-labels of thymidine-^{3}H. The lines represent theoretical Poisson distributions for the observed average mitochondrial grain counts ($\bar{X}$).

DISCUSSION

Today there is ample evidence for the existence of mitochondrial DNA (11, 24). Electron micrographs (19, 11, 25, 30), electron microscope radioautographs (1, 22, 10), and the resistance of mitochondrial DNA to DNase digestion in cell homogenates (7, 16, 23, 29) all indicate that the DNA is within the matrix area of mitochondria. Results obtained with CsCl density gradient centrifugation indicate that mitochondrial DNA often has a different base ratio than nuclear DNA from the same cell (7, 2, 16, 17, 23, 26). Reich and Luck (18) have reported at least two types of DNA associated with mitochondria in *Neurospora*. Moustacchi and Williamson (9) and Mounolou et al. (8) have also found variations in the buoyant density of yeast mitochondrial DNA which are correlated with cytoplasmic mutations.

Isolated mitochondrial DNA shows an increase in buoyant density (17, 24, 23) and increase in optical density (26, 23) when heat-denatured. This indicates that this DNA is double stranded. Recent electron micrographs of extracted DNA (12, 21, 27) have shown that mitochondrial DNA's from several vertebrate sources have a circular configuration whereas nuclear DNA's from the same cells are linear. The circles have a length of about 5 μ which is equivalent to a molecular weight of about 10×10^6. Estimates of the amount of DNA per mitochondrion range from 20 to 240×10^6 daltons (26, 23, 12). The wide range in DNA content may represent species differences or inac-

curacies due to the extraction procedure. The data do indicate, however, that there are several DNA molecules per mitochondrion.

There is good evidence for mitochondrial DNA in *Tetrahymena* (15, 22, 23). Electron microscope radioautographs reported by Stone and Miller (22) showed 92% of the cytoplasmic grains over mitochondria and 97% of the grains within 1.0 μ of mitochondria. This evidence indicates that the only detectable cytoplasmic DNA in *Tetrahymena* is mitochondrial DNA. The present experiments also indicate the mitochondrial localization of this cytoplasmic DNA (15). The number of cytoplasmic grains at greater than 1 μ from mitochondria were not above background, except when nuclei were fragmented or when improper fixation permitted swelling and rupture of mitochondrial membranes. Since the DNA from these ruptured organelles did remain on slides throughout the radioautography procedure, nonmitochondrial cytoplasmic DNA should have been detected with the squash technique. Labeling of mitochondria occurred at all times throughout the cell growth cycle, even in the absence of nuclear DNA synthesis. This finding suggests that DNA is not only associated with mitochondria, but is synthesized by this organelle as well. During the G_2 interphase of *Physarum*, Evans (4) has observed synthesis of a satellite DNA which is believed to be of mitochondrial origin.

Although some labeling of *Tetrahymena* mitochondria occurred at all stages of the cell cycle, a significant increase in labeling was found during the period of macronuclear DNA synthesis. This might indicate a common trigger which stimulates both mitochondrial and macronuclear DNA syntheses. The difference in mitochondrial-labeling rate between G and S periods might indicate instead that the extensive amount of macronuclear DNA synthesis quickly depletes intracellular nucleoside and nucleotide pools. The same rate of mitochondrial DNA synthesis would then produce a higher label density because a higher proportion of externally supplied thymidine-^{3}H would be incorporated into DNA.

Statistical analysis demonstrated a Poisson distribution of the mitochondrial label after incorporation of thymidine-^{3}H for one cell cycle duration. Thus, all mitochondria appear to incorporate DNA percursors and increase their content of DNA during each generation. The data indicate that the cytoplasmic DNA of *Tetrahymena* is synthesized by all mitochondria in the cell and is a characteristic component of mitochondrial structure.

The stability of the mitochondrial DNA was demonstrated by the conservation of label within the population of mitochondria during four generations of logarithmic growth (15). The stability of the *Tetrahymena* mitochondrial DNA has been confirmed for three generations by Stone and Miller (22). Mitochondrial DNA was not transported outside mitochondria, as indicated by extremely low radioautograph background. Similarly, the clearly visible surface mitochondria of living *Tetrahymena* do not migrate throughout the cell or show the fusion-fission phenomenon seen by Frederic and Chèvremont (5) in tissue culture cells. Stone and Miller (22) reported that they never observed structural associations between mitochondria and nuclei in electron micrographs. Transfer of DNA by direct fusions of mitochondria to mitochondria or mitochondria to nuclei, therefore, is unlikely. One other possible mechanism of label exchange between mitochondria might involve the degradation of labeled DNA to nucleotides. The nucleotides could enter a common pool and be reused for DNA synthesis. Nucleotides would be washed from cells during the slide preparation, and radioactivity within this pool would not be recorded in radioautographs. If prelabeled cells, starved to remove any nucleotide pools, were grown in nonradioactive medium, this type of metabolic turnover would involve cycling of a fixed number of radioactive thymine residues which would be constantly diluted by the addition of nonradioactive DNA precursors. The result would be a gradual loss in total mitochondrial label in the cell population. Since the amount of mitochondrial label remained constant for four generations, the mitochondrial DNA of *Tetrahymena* is apparently stable for at least this period of growth.

Experiments with prelabeled cells have also shown that the label remained randomly distributed among all mitochondria in a cell during four generations' growth in unlableled medium. During this time, the mitochondrial population would have increased by a factor of 16. The labeled mitochondrial DNA molecules, therefore, must also have increased their distribution by a factor of 16. For reasons discussed above, it is unlikely that mitochondrial DNA is degraded or exists free of the mitochondrial structure. The only way a mitochondrion could, therefore, distribute its label to 16 other mitochondria would be to be-

come these 16 organelles. On the basis of the experimental conditions used in the present study, we can only conclude that mitochondria arise by the growth and division of preexisting mitochondria.

The random assortment of labeled mitochondrial DNA suggests some characteristics of this molecule. If there were only one DNA molecule per mitochondrion, this distribution would indicate a very high degree of "crossing over" or fragmentation. In the absence of any evidence for such "crossing over," it is reasonable to propose that the mitochondrial DNA molecule fragments very little during four generations of growth and that each mitochondrion contains a minimum of $2^{(n-1)}$ or eight molecules of double-stranded DNA. A mitochondrion might then have several copies of the same DNA molecule. If there is no intramitochondrial mechanism for equal DNA dispersal, each daughter mitochondrion is more likely to receive a copy of any genetic information coded within the molecule if several identical molecules are available before division.

Although it is not presently known whether mitochondrial DNA contains genetic information, two properties of genetic material have been demonstrated by the present experiments: the mitochondrial DNA seems to be stable, and is effectively distributed among daughter mitochondria. The results also indicate that DNA is synthesized by mitochondria.

In view of the large body of literature on the origin of mitochondria, some comparisons with the present results are needed. Three theories on the origin of mitochondria exist: (*a*) de novo formation from newly synthesized material, (*b*) differentiation of cellular "microbodies" or nonmitochondrial membranes, and (*c*) mitochondrial growth and division (for review, see 13).

The present experiments were designed for a population of logarithmically growing cells. Mitochondria in these cells were followed chronologically by means of a structurally stable molecular label. Most published studies relating to the origin of mitochondria differ in these two experimental conditions and thereby make comparisons difficult. In one similar experimental design, however, Luck (6) used choline-^{3}H to label lipids and obtained essentially the same mitochondrial grain distributions as the present experiments. Luck found that *Neurospora* showed no loss of total mitochondrial label during three mass doubling cycles in the mycelium. During this time, grains over isolated mitochondria showed a Poisson distribution in radioautographs. Similarly, short pulse labels showed mitochondrial grain counts which closely fit a Poisson distribution. The persistent random distribution of grains throughout the entire mitochondrial population indicates that lipid material already present in the mycelium is used in the formation of new mitochondria. Since unlabeled mitochondria were not found, de novo formation of mitochondria from all newly synthesized material is not occurring. It is not clear, however, that the choline-^{3}H once incorporated into a mitochondrion would remain uniquely associated with that mitochondrion and its progeny. It should be stressed that choline-^{3}H labels the entire lipid fraction and thus all the membranes of the mycelium. The electron micrographs of Robertson (20) show clear unit membrane interconnections between mitochondria and the endoplasmic reticulum. Because of the dynamic nature of cellular membranes, it is likely that unit membrane incorporates new material and perhaps exchanges material at many sites along its surface. Mitochondria could then be assembled from previously synthesized membrane subunits at any site within the cell and show a randomization of choline label. The primary question of the origin of mitochondria is not the time or the site at which membrane molecules are synthesized, but rather the site at which the mitochondrial membranes are assembled. On the basis of the general membrane label used in Luck's experiments, it is impossible to distinguish between mitochondrial division and "microbody" origin from previously synthesized membrane.

The present experiments, then, have the advantage of using a mitochondrial marker which is specific for mitochondria and seemingly not in equilibrium with any other cytoplasmic component. The data do not fit any mechanism of de novo or "microbody" formation of mitochondria. The results do indicate, however, that under the conditions of logarithmic growth the mitochondria of *Tetrahymena pyriformis* assemble their own new structure and divide.

This work was supported in part by contract AT-(40-1)-2730 with the United States Atomic Energy Commission, and by a contract between the Institute of Molecular Biophysics, Florida State University and the Division of Biology and Medicine, United States Atomic Energy Commission. Funds were also provided by the United States Public Health Service in fellowships GF-17,709 and 5 F1

GM-17,709-02 from the Division of General Medical Sciences.

Portions of this work were submitted by J. A. Parsons in partial fulfillment of the requirements for the Ph.D. degree, Florida State University, August, 1964. The work was presented at the American Society for Cell Biology meetings, November 11–13, 1964, Cleveland, Ohio.

Preparation of this manuscript was supported by a grant from the National Science Foundation to J. A. Parsons and by contract W-31-109-ENG-78 with the United States Atomic Energy Commission (Report number C00-78-169).

Thanks are extended to Dr. A. Gib DeBusk and Dr. James R. Fisher for valuable discussions and suggestions, to Mr. Richard Cornell for advice on statisical procedures, and to Dr. Oddvar F. Nygaard for helpful suggestions in the preparation of the manuscript.

Received for publication 3 April 1967, and in revised form 17 January 1968.

REFERENCES

1. BELL, P. R., and K. MÜHLETHALER. 1964. *J. Cell Biol.* **20**:235.
2. DUBUY, H. G., C. R. T. MATTERN, and F. L. RILEY. 1965. *Science.* **147**:754.
3. ELLIOTT, A. M., and R. E. HAYES. 1954. *Biol. Bull.* **105**:269.
4. EVANS, T. E., 1966. *Biochem. Biophys. Res. Commun.* **22**:678.
5. FREDERIC, J., and M. CHÈVREMONT. 1952. *Arch. Biol.* **63**:109.
6. LUCK, D. J. L. 1963. *J. Cell Biol.* **16**:483.
7. Luck, D. J. L., and E. Reich. 1964. *Proc. Natl. Acad. Sci.* **52**:931.
8. MOUNOLOU, J. C., J. JAKOB, and P. P. SLONIMSKI. 1966. *Biochem. Biophys. Res. Commun.* **24**:218.
9. MOUSTACCHI, E., and D. H. WILLIAMSON. 1966. *Biochem. Biophys. Res. Commun.* **23**:56.
10. MUCKENTHALER, F. A., and A. P. MAHOWALD. 1966. *J. Cell Biol.* **28**:199.
11. NASS, M. M. K., S. NASS, and B. A. AFZELIUS. 1965. *Exptl. Cell Res.* **37**:516.
12. NASS, M. M. K. 1966. *Proc. Natl. Acad. Sci.* **56**: 1215.
13. NOVIKOFF, A. B. 1961. *In* The Cell. J. Brachet and A. E. Mirsky, editors. Academic Press, Inc., N. Y. **2**:299.
14. PARSONS, J. A. 1964. *J. Cell Biol.* **23**:70A (Abstr.).
15. PARSONS, J. A. 1965. *J. Cell Biol.* **25**:641.
16. PARSONS, J. A., and R. C. DICKSON. 1965. *J. Cell Biol.* **27**:77A (Abstr.).
17. RABINOWITZ, M., J. SINCLAIR, L. DESALLE, R. HASKELKORN, and H. H. SWIFT. 1965. *Proc. Natl. Acad. Sci.* **53**:1126.
18. REICH, E., and D. J. L. LUCK. 1966. *Proc. Natl. Acad. Sci.* **55**:1600.
19. RIS, H., 1962. In 5th International Congress for Electron Microscopy, Philadelphia. S. S. Breese, Jr., editor. Academic Press, Inc., N. Y. 2, XX.
20. ROBERTSON, J. D. 1961 Proceedings of the 4th Neurochemical Symposium. 497.
21. SINCLAIR, J. H., and B. J. STEVENS. 1966. *Proc. Natl. Acad. Sci.* **56**:508.
22. STONE, G. E., and O. L. MILLER JR., 1965. *J. Exptl Zool.* **159**:33.
23. SUYAMA, Y., and J. R. PREER, JR. 1965. *Genetics.* **52**:1051.
24. SWIFT, H. 1965. *Am. Naturalist.* **99**:201.
25. SWIFT, H., B. J. ADAMS, and K. LARSEN. 1964. *J. Roy. Microscop. Soc.* **83**:161.
26. TEWARI, K. K., J. JAYARAMAN, and H. R. MAHLER. 1965. *Biochem. Biophys. Res. Commun.* **21**:141.
27. VAN BRUGGEN, E. F. J., P. BORST, G. J. C. M. RUTTENBERG, M. GRUBER, and A. M. KROON. 1966. *Biochim. Biophys. Acta.* **119**:437.
28. VAN TUBERGEN, R. P., and R. B. SETLOW. 1961. *Biophys. J.* **1**:589.
29. WINTERSBERGER, E. 1966. *Biochem. Biophys. Res. Commun.* **25**: 1.
30. YOTSUYANAGI, Y. 1965. *Collog. Soc. Française Microscop. Electronique.* 79. (Abstr.).