

MACROMOLECULAR SYNTHESSES RELATED TO THE REPRODUCTIVE CYST OF *TETRAHYMENA PATULA*

P. R. GABE and L. E. DE BAULT

From the Departments of Zoology, and Psychiatry and Anatomy, College of Medicine, University of Iowa, Iowa City, Iowa 52242

ABSTRACT

Macromolecular syntheses in encysted *Tetrahymena patula* were studied using Feulgen fluorescence cytophotometry, autoradiography, and inhibitors of RNA and protein synthesis. Cycloheximide significantly depressed protein synthesis and D-actinomycin effectively blocked RNA synthesis. Under these conditions, the cells within the cyst were unable to divide. Both cytophotometric measurements and autoradiographic data with tritiated thymidine show that DNA synthesis does not occur during the encystment divisions. Excysted cells placed in nutrient broth medium showed a prolonged generation time after the first cell growth cycle, and by the third generation the mean DNA content per cell was almost triple that of starved excysted cells. These findings indicate that (a) the encystment divisions require RNA and protein synthesis, which are apparently effected through turnover, (b) the encystment division cycles occur in the absence of DNA synthesis, and (c) excysted cells placed in culture medium may go through more than one DNA replication per cell cycle.

INTRODUCTION

In a previous study, a method was described for obtaining reproducibly high yields (~90%) of encystment in the ciliated protozoan *Tetrahymena patula* (Gabe, 1973). Cells placed under starvation conditions elaborate a gelatinous cystic envelope within which they undergo two or three rapid divisions producing four or eight small daughter cells. The divisions within a cyst are synchronous and occur in the absence of any apparent growth. Thus, after three divisions, each daughter cell is approximately one-eighth the size of its precystic progenitor. Further, the generation time for cells in the reproductive cyst is about one-fourth the average generation time of log-phase cells in culture medium.

In the present investigation the relationship of macromolecular syntheses to this unusual mode of

cellular reproduction has been studied. The first objective was to determine whether DNA, RNA, or protein synthesis occurs while the cells are under encystment conditions. Using autoradiography in conjunction with the appropriate precursors, it was found that RNA and protein are synthesized before and during the cystic division cycles, but the data from autoradiography and Feulgen cytophotometry indicate that DNA is not. The second objective was to determine whether the synthesis of RNA and protein are necessary for the cystic divisions. The studies with inhibitors indicate that these syntheses are required. Additional data are presented concerning the macronuclear DNA content of excysted cells which were placed in nutrient broth medium and allowed to grow and reproduce. It was found that the mean DNA content per

cell regulates upward over the course of successive cell growth cycles. A short account of part of this work has been published elsewhere (Gabe, 1971).

MATERIALS AND METHODS

Preparation of Cells for Encystment

An axenic culture of *T. patula* L-FF, an amicro-nucleate strain, was obtained from Dr. Norman E. Williams, Department of Zoology, University of Iowa. The culture medium, methods of maintenance, and conditions for encystment were the same as those reported in a previous study (Gabe, 1973).

The method for obtaining encystment (Gabe, 1973) involved a slight modification of the original procedure devised by Nachtwey and Dickinson (1967) for single-cell studies on *Tetrahymena pyriformis*. Briefly, the washed cells were pipetted individually into 1–3- μ l drops of distilled water ("microdrops") set up on the inside bottom surface of a glass Petri dish (100 cells per dish). The drops were covered with a thin layer of light paraffin oil to prevent desiccation. Under these conditions about 90% of the cells undergo the first division within 3–6 h and subsequent divisions take place at about 3 h intervals.

Incorporation of ^3H -Labeled Thymidine, Uridine, and Leucine

Methyl-labeled thymidine (3.0 Ci/mmol; Schwarz Bio Research Inc., Orangeburg, N. Y.) was used for the detection of DNA synthesis; [^3H]uridine (21.7 Ci/mmol; International Chemical and Nuclear Corp. [ICN], Chemical and Radioisotope Div., Irvine, Calif.) for RNA synthesis; and [4,5- ^3H]leucine (56 Ci/mmol; Schwarz Bio Research Inc.) for protein synthesis. Cycloheximide (actidione; ICN, Nutritional Biochemicals Div., Cleveland, Ohio) and D-actinomycin (a gift from Merck Chemical Div., Merck and Co., Inc., Rahway, N. J.) were used as inhibitors of protein and RNA synthesis, respectively.

For incorporation studies, an equal volume of isotope, at twice the working concentration, was added to the cells 2 h after they were placed in microdrops. To test the effect of inhibitors on cell division and RNA and protein synthesis, cells were pipetted directly into microdrops containing D-actinomycin or cycloheximide, respectively; [^3H]uridine or [4,5- ^3H]leucine were added 2 h later. Cells were incubated in the isotopic solutions for 1 h, then removed, transferred through three changes of distilled water, and air dried on subbed slides (Caro, 1964).

Autoradiography

After fixation in 3:1 ethanol-acetic acid for 30 min the slides were hydrated and extracted in cold (5°C)

5% trichloroacetic acid for an additional half hour. After transfer through three changes of 70% alcohol, the slides were air dried. Autoradiography was done according to the procedures outlined by Prescott (1964). Slides were exposed in the dark for 14 days. Cells were examined after autoradiography at $\times 400$ and grain counts were made with the aid of an ocular grid.

Fluorescence Cytophotometry

The DNA contents of encysted and excysted cells were determined by fluorescence cytophotometry. All DNA values were from macronuclei. All stages of cystment were dried down on the same slide in each experiment. Cells were fixed in 3:1 ethanol-acetic acid for 30 min, transferred to absolute alcohol, and coated with a thin layer of celloidin (about 5 drops/50 ml of 50:50 ether-alcohol; "Tissue-Imbedding Solution," Randolph Products Co., Carlstadt, N. J.). Staining was done using the Feulgen procedure of Elftman (1959). After Feulgen staining, the relative amount of DNA per cell was measured microfluorometrically. For these measurements the Leitz MPV fluorometer (Ernest Leitz gmbh, Wetzlar, Germany) was used in an optical arrangement that allowed successive and/or simultaneous illumination with transmitted and incident light and was similar to that described by F. Ruch (1970). Since the pararosaniline dye of the Schiff-reagent has a low fluorescence efficiency, high levels of exciting irradiation are required to produce detectable amounts of red fluorescence. This was achieved by illuminating the specimen from the top with a Ploem vertical illuminator, which uses the objective in reverse to focus the exciting light onto the specimen at a very high flux density (Ploem, 1967). The specific optical arrangement in the Leitz MPV fluorometer used in the measurements utilized a high pressure xenon lamp XBO 150 W (Osram, Berlin) in combination with an interference heat-protecting filter, a red absorbing filter 5 mm BG38, and a Hg546 interference filter to produce a near-monochromatic light source at 546 nm. This intense green light was reflected and focused onto the specimen by a dichroic mirror (Leitz TK580 and a F1 Oel 54 \times objective, respectively). The induced red fluorescence was then collected by the objective lens, passed through the dichroic mirror and a K580 barrier filter (both of which transmit red light), the area of the nucleus optically isolated by the MPV diaphragm, and the image of the nucleus projected onto the photomultiplier S-20 type 9558AQ with a quartz window. Only a narrow band of the red spectrum was used for the measurement (Bohm and Sprenger, 1968) and was selected by a 60 mm interference wedge filter set at 660 nm. Thus, fluorescence intensities at 660 nm were recorded as photomultiplier voltage output and were proportional to the DNA content.

RESULTS

Autoradiographic Detection of Protein, RNA, and DNA Synthesis

The results from autoradiographic assays for the incorporations of tritium-labeled precursor molecules into protein or RNA indicate that the synthesis of both takes place during the cystic division cycles. The data from a representative experiment for each are shown in Table I. The data are expressed as grain counts per 100 μm^2 of cell surface corrected for background labeling. The incorporation of [^3H]leucine and [^3H]uridine into macromolecular materials was effectively blocked by 5 $\mu\text{g}/\text{ml}$ cycloheximide or 30 $\mu\text{g}/\text{ml}$ D-actinomycin, respectively (Table I). In addition, the drugs completely suppressed cell division for at least 18 h (the first division normally occurs at 3–5 h). Addition of either cycloheximide or D-actinomycin just before or during the first division did not affect their progress through the first division but the cells did not divide a second time. If the drugs were added just after the first division

the cells did not divide again. The same effect was observed on cells at the four-celled stage; drugs added late in the first cystic cell cycle had no effect on the second cystic division but the cells did not divide a third time. If the concentration of D-actinomycin was lowered to 15 $\mu\text{g}/\text{ml}$ and added at the beginning of the experiment, many of the cells initiated cleavage within the 6 h period but remained furrowed without completing division. The effect was not observed using lower concentrations of cycloheximide; those cells that entered division completed the process normally.

Previously it was reported that neither RNA nor protein synthesis is necessary for cyst wall formation in *T. patula* (Gabe, 1971). This was based upon the observation that cells incubated in the presence of the drugs exhibited a blue halo, thus indicating the presence of a cyst wall, when exposed to dilute solutions of aqueous Alcian blue 8GX. Although in later stages of encystment the cyst wall can be easily demonstrated after staining (Gabe, 1973), it was subsequently discovered that in the early stages of encystment the dye induces

TABLE I
The Effect of D-Actinomycin and Cycloheximide on RNA and Protein Synthesis, Respectively, and on Cell Division in Encysting Tetrahymena patula

Agents	GC	$\pm\text{SE}$	CV%	% Division	N
[^3H]Uridine (10 $\mu\text{Ci}/\text{ml}$)	44.00	4.70	47.75	90	20
[^3H]Uridine + D-actinomycin (30 $\mu\text{g}/\text{ml}$)	3.44	0.95	1.38	0	25
[^3H]Leucine (2 $\mu\text{Ci}/\text{ml}$)	14.00	2.20	7.85	91	25
[^3H]Leucine + cycloheximide (5 $\mu\text{g}/\text{ml}$)	1.08	0.14	6.48	0	25

More than 100 cells were used for each experiment. Cells were exposed to the appropriate isotope between the second and third hours after being set up for encystment in water or in water plus inhibitor, and then prepared for autoradiography.

GC = mean grain counts per 100 μm^2 of cell surface with background count subtracted.

SE = standard error of mean.

CV% = coefficient of variation.

N = number of cells used for grain count determinations. Percent division was determined from the remaining cells at 6 h.

the cells to release copious amounts of a gelatinous material which also takes up the stain. Since both the gelatinous exudate and the cyst wall material are stained by Alcian blue, it is impossible to distinguish between the two. Thus, the claim that cyst wall material is produced in the absence of protein or RNA synthesis must be retracted until it can be demonstrated that the material that stains blue is actually the cyst wall and not something that is produced by the cell in response to toxic materials in the medium (Bresslau, 1921).

Cells pipetted directly into microdrops containing [³H]thymidine (5–50 μ Ci/ml) and prepared for autoradiography after the first, second, and third encystment divisions showed no concentration of label over the macronucleus. There was some residual cytoplasmic label, however, this may have been due to mitochondrial DNA synthesis or incorporation into molecules other than DNA (Wand et al., 1967). Thus, no DNA synthesis could be detected in encysted cells between successive divisions by this method

DNA Cytophotometry of Encysted Cells

The evidence from Feulgen fluorescence cytophotometry confirms the above results obtained by autoradiography using [³H]thymidine. Measurements obtained from cells after the first, second, and third divisions, respectively, showed a progressive decline in the mean DNA content (Table II; Fig. 1). Although every attempt was made to select large cells of uniform size, a wide range of DNA contents was found among precystic cells. After the first division, there was an approximate 50% reduction in the mean DNA content. At the

four-celled stage, however, the mean DNA content was less than what one would expect if the mean DNA content of cells at the two-celled stage had been halved after the second cystic division. It is suspected that this may be due to some unrecognized error in the sampling procedure.

On the other hand, the mean DNA content of cells from eight-celled cysts was higher than would be expected if the original mean precystic value had been halved at each previous generation (Table II). This might indicate that eight-celled cysts are generated from precystic cells whose DNA contents were rather high relative to the mean and that terminal four-celled cysts are derived from precystic cells that possessed low DNA contents. To test this hypothesis cells were set up for encystment in the usual way and allowed to progress to the four-celled stage. A portion of the four-celled cysts, excysted cells from four-celled cysts, and cells that were permitted to divide a third time to form eight-celled cysts were removed from the microdrops and prepared for cytophotometry. According to the hypothesis, cells in four-celled cysts would show a higher mean DNA content and a larger standard deviation than that of cells excysted from four-celled cysts, since a portion of the sample of four-celled cysts would represent those former precystic cells that had relatively high DNA contents and were, therefore, destined to produce eight-celled cysts. Those which progressed to eight-celled cysts would be expected to have DNA contents not much lower than those excysted from four-celled cysts, since although they underwent an additional division, they are postulated to come from the

TABLE II
The Macronuclear DNA Content of Cells at Successive Stages of Cystment

Exp. no.	Precystic cells				Two-celled cysts				Four-celled cysts				Eight-celled cysts				Encysted cells			
	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N
1	648	31	35	52	325	13	30	57	121	5	32	51	87	3	24	44	93	4	35	54
2*	714	41	40	48	355	22	33	29	153	6	32	58	109	3	22	64	118	4	27	72
3	475	19	29	50	228	19	27	11	96	6	31	26	77	3	17	23	85	4	28	33
4	606	19	32	100	299	17	34	35	105	4	31	65	73	1	16	93	83	2	22	66

\bar{X} = mean.

SE = standard error of mean.

CV% = coefficient of variation.

N = number of cells.

* Data presented as a frequency distribution histogram in Fig. 1.

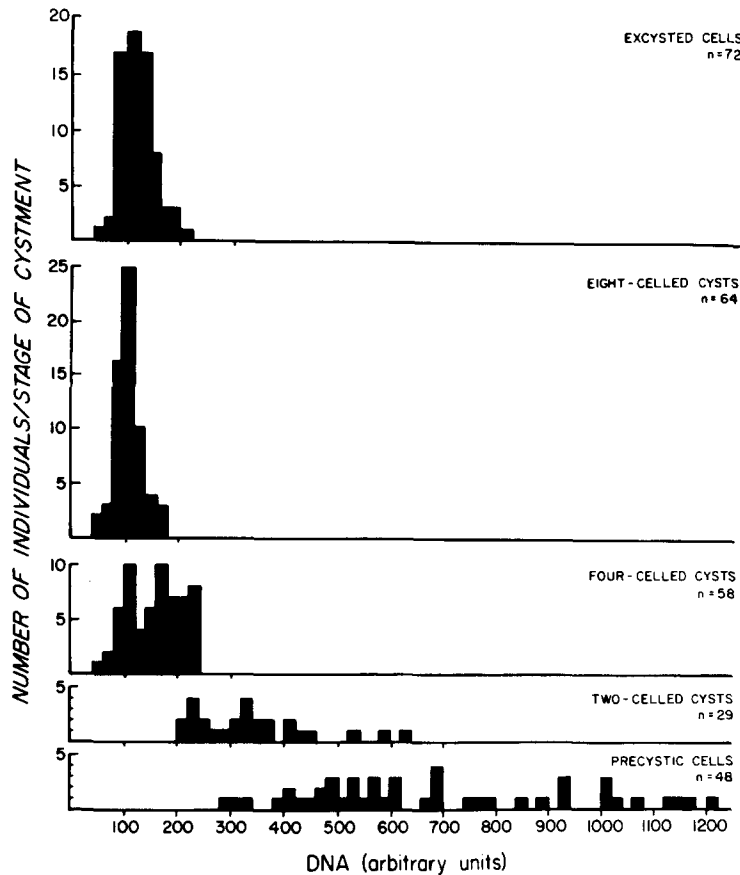


FIGURE 1 Histogram of the results of cytophotometric measurements of the DNA content of *T. patula* during cystment. Abscissa = DNA contents in arbitrary units. Ordinate = number of individuals at each stage.

upper end of the distribution of DNA contents in four-celled cysts. The means reported in Table III are in agreement with this interpretation. Cells in four-celled cysts represent a population whose range of DNA values are considerably broader than either of the other categories also as predicted. An F test for variance ratios showed that their DNA contents were significantly more variable than cells in eight-celled cysts ($P < 0.001$) and excysted cells from four-celled cysts ($P < 0.01$).

The unselected samples of excysted cells (Table II) consistently showed a higher mean DNA content and greater variance than cells at the eight-celled stage. These differences can be at least partly accounted for by the significantly higher ($P < 0.001$) and more variable DNA content of the cells excysted from four-celled cysts (Table III). Excysted cells from eight-celled cysts were found to have DNA contents that were

not significantly different from those of cells contained in eight-celled cysts within 95% confidence limits ($n = 64$ and 40 , respectively). Since about 15–20% of the precystic cells produce terminal cysts containing five, six, and seven cells (Gabe, 1973), these may also be expected to contribute some variability in the DNA values of excysted cells. The results from cytophotometric determinations made on these cells are shown in Table IV. Because their numbers were few, the cells excysted from these cysts were grouped together under the heading “odd-membered cysts.” For comparison, cells excysted from four- and eight-celled cysts were also measured. The data, shown in Table IV, were obtained from three separate experiments. The mean DNA content of cells excysted from eight-celled cysts was normalized to 100 arbitrary units and the values obtained from each class were adjusted to this value. These data (Table IV) indicate that the

TABLE III
A Comparison of the Mean DNA Contents of Cells in Four-Celled Cysts, Excysted Cells from Four-Celled Cysts, and Cells in Eight-Celled Cysts

Cells in four-celled cysts				Excysted cells from four-celled cysts				Cells in eight-celled cysts			
\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N
155	6.6	32.5	58	123	4.3	18.3	28	103	2.5	17.3	53

\bar{X} = mean.
SE = standard error of mean.
CV% = coefficient of variation.
N = number of cells.

TABLE IV
The Mean DNA Content and Coefficient of Variation of Cells Excysted from Four-Celled Cysts, Odd-Membered Cysts (i.e., Cells Derived from Five, Six, and Seven-Celled Cysts), and Eight-Celled Cysts

Experiment	Cells from four-celled cysts				Cells from odd-membered cysts (5, 6, and 7)				Cells from eight-celled cysts			
	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X} *	\pm SE	CV%	N
1	120	6.12	30	35	112	3.40	25	66	100	3.50	21	33
2	116	6.40	26	22	105	4.68	28	39	100	2.75	16	36
3	133	4.58	18	28	—	—	—	—	100	1.30	7	30
Pooled (1, 2, 3)	123	3.41	26	85	109	2.76	26	105	100	1.59	16	99

* The data from each experiment were normalized by adjusting the mean DNA content of cells excysted from eight-celled cysts to 100 arbitrary units. Excysted cells derived from eight-celled cysts were chosen for the standard because they showed the least intraclass variation.

\bar{X} = mean.
SE = standard error of mean.
CV% = coefficient of variation.
N = number of cells.

mean DNA content of cells excysted from eight-celled cysts is much less than that of cells excysted from four-celled cysts ($P < 0.001$) and slightly less than that of cells excysted from odd-membered cysts ($P < 0.02$). Thus, the consistently higher and more variable DNA contents observed in the unselected samples of excysted cells as compared to the DNA contents of cells at the eight-celled stage of encystment (Table II) can be largely accounted for by the higher and more variable DNA contents observed in cells excysted from four-celled cysts (Table III) and to a lesser extent to the cells excysted from odd-membered cysts (Table IV).

DNA-Cytophotometry of Excysted Cells

When excysted cells from eight-celled cysts were placed in microdrops of culture medium,

they grew and divided in about 10–12 h. Over the next two generations, some of the cells showed a prolonged generation time (in some cases up to 24 h). Cytophotometric determinations were made on cells selected at random times of the inter-division periods of the first three cell generations. Thus, the DNA contents of the excysted cells shown in Fig. 2 represent a mixture of G_1 , S, and G_2 cells. Some of these cells showed a marked increase in their DNA contents so that by the third generation, the mean was triple that of starved excysted cells (Table V). From these data, it is clear that some of the cells undergo more than one DNA replication per cell cycle.

DISCUSSION

Large stationary phase *T. patula* placed in non-nutrient medium may undergo two or three rapid divisions within the temporary confines of a

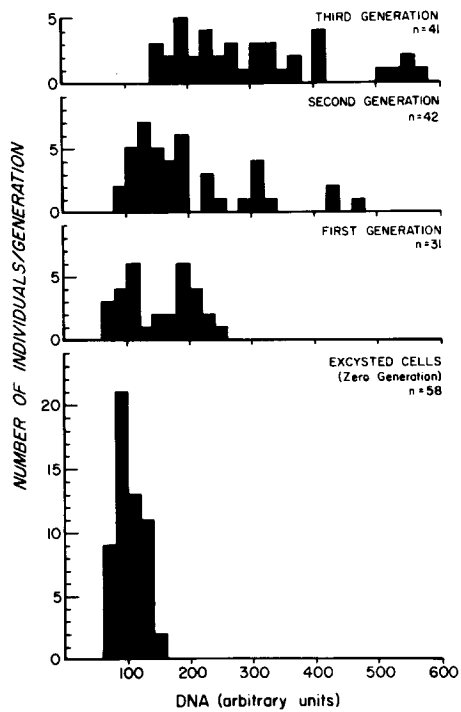


FIGURE 2 Histogram of the results of cytophotometric measurements of the DNA content of excysted *T. patula* during growth in culture medium. Abscissa = DNA content in arbitrary units. Ordinate = number of cells per generation. Pooled data from three separate experiments, normalized to the mean DNA content of excysted cells (= 100 arbitrary units).

fragile cyst wall, resulting in usually four or eight small daughter cells. It is clear that each division produces progressively smaller cells; however, it is not certain that the division cycles occur in the complete absence of growth. Accurate volume measurements were not possible because the encysted cells were seldom entirely quiescent and their movement often caused them to appear distorted due to the restrictive confines. However, since the conditions that induce encystment also preclude net synthesis of protein, it would seem that growth is unlikely.

Autoradiographic studies indicate that [³H]-uridine and [³H]leucine are incorporated into RNA and protein, respectively (Table I). If the same precursors are added in combination with the appropriate inhibitors, RNA or protein synthesis is blocked and the cells do not divide. Similar data on the synthesis of macromolecules required for the production of the cyst wall were not obtained because at present there is no reliable method for detecting early stages of cyst wall formation (cf. Results).

These findings show that the events associated with the reproductive cyst in *T. patula* are initially triggered by starvation, and suggest that the degradation of endogenous protein and RNA provides the necessary precursors for the synthesis of division-related macromolecules.

The results obtained with [³H]thymidine, using autoradiography, and the data from cytophotom-

TABLE V
The Mean DNA Content of Excysted *T. patula* before and after Refeeding

Exp. no.	Excysted cells*				First generation†				Second generation‡				Third generation‡			
	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N	\bar{X}	\pm SE	CV%	N
1	89.9	4.3	21	19	165.6	7.5	17	14	265.9	26.5	33	11	281.0	32.3	23	4
2	111.6	6.5	21	13	231.0	17.3	13	3	218.8	36.5	5	9	421.9	40.0	34	13
3	119.7	5.2	22	26	126.0	11.8	35	14	180.0	8.4	23	22	301.9	23.4	38	24
Pooled§ (1, 2, 3)	100.0	2.8	21	58	150.0	9.4	35	31	197.7	14.9	49	42	297.7	18.6	40	41

* Excysted cells from eight-celled cysts were collected and divided into two groups. One group was prepared for cytophotometry; individuals of the other group were pipetted into microdrops of culture medium and allowed to grow and divide.

† One of each pair of daughter cells was selected for cytophotometry at random times of the cell cycle.

‡ Normalized to mean excysted cells (= 100 arbitrary units); data taken from Fig. 2.

\bar{X} = mean.

SE = standard error of mean.

CV% = coefficient of variation.

N = number of cells.

etry (Table II, Fig. 1) indicate that DNA synthesis does not occur during the encystment division cycles. A similar phenomenon has been described in the ciliate, *T. pyriformis*. This cell, which does not form a cyst, has been used extensively for division synchrony studies. In this case, two divisions are effected with no detectable DNA synthesis (Lowy and Leick, 1969; Jeffery et al., 1970) after the cells have accumulated an excess of DNA during the synchronizing treatments (Scherbaum et al., 1958 *b*; Hjelm and Zeuthen, 1967; Andersen et al., 1970). However, in *T. patula*, division without previous DNA synthesis is regularly performed in the reproductive cyst as a natural event in its life history.

The data presented in Fig. 2 show that excysted refeed *T. patula* accumulate extra replicated DNA during vegetative reproduction by sometimes undergoing more than one S period per cell cycle. Successive DNA replications have also been observed during the normal cell cycle (Kimball and Prescott, 1962; Uspenskaja and Ovchinnikova, 1966; Cleffman, 1968) and after experimentally blocking cell division (Shepard, 1965; Jerk-Dziadosz and Frankel, 1970; Jeffery et al., 1970; Jeffery, 1972) in other ciliates. Although the cells were only followed over three generations, these data suggest that if they had been permitted to multiply longer many of them would have undergone additional replications eventually leading to DNA values comparable to those of precystic cells.

A number of observations in the present study are consistent with the notion that there is a limit to the number of divisions which can occur in the absence of DNA synthesis. The number of divisions is that which produces cells whose DNA contents are somewhere in the region of the mean value at the four-celled stage. Cells which possess more DNA than this may divide once more, but cells with lower DNA values probably do not. This would be consistent with the observations that (*a*) the mean DNA content of eight-celled cysts is more than one-eighth that of precystic cells, (*b*) the variance of DNA values drops markedly from the four-celled to the eight-celled stage, and (*c*) the mean DNA content of a randomly selected population of recently excysted cells is higher than the DNA content of cells within eight-celled cysts.

The occurrence of five-, six-, and seven-celled cysts is difficult to interpret, nevertheless two possible explanations may be considered: (*a*) macronuclear extrusion—large macronuclear extrusion bodies were observed in some cells at the

two-celled cyst stage (unpublished observation), or (*b*) unequal macronuclear division—unequal partitioning of the macronucleus beyond the usual slight variations that occur during cell division. In either case the daughters would ultimately possess detectable differences in their DNA contents. Assuming that there is a lower limit of DNA which precludes further cell division, one or more of the cells in a cyst will reach this limit before the others because they have less DNA. The first hypothesis seems unlikely since recent evidence indicates that macronuclear extrusion serves to regulate the amount of DNA below certain upper limits by eliminating the excess (Shepard, 1965; Cleffman, 1968). Scherbaum et al. (1958 *a*), Shepard (1965), and Cleffman (1968) independently noted that the amount of DNA extruded was proportional to the amount of DNA in the macronucleus from which the extrusion body originated. Therefore, the extrusion bodies found in cells from two-celled cysts most likely represent excessive DNA that was synthesized before encystment. In regards to the second hypothesis, excessive unequal macronuclear division is known to occur in *T. pyriformis* (Cleffman, 1968), in the “amacronucleate” mutant of *Paramecium aurelia* (Kimball, 1967), and in other ciliates (Raikov, 1968). In *T. pyriformis*, Cleffman found that the daughter that received the lesser quantity of DNA sometimes underwent two successive replications before dividing again. In the reproductive cyst of *T. patula*, however, there is no DNA synthesis. The daughter receiving the smaller macronuclear portion, therefore, may divide again, or it may already have reached its limit and not divide further; meanwhile, its sister cell, containing the larger share, could continue to multiply in the absence of DNA synthesis until it too reaches this limit.

From the above discussion it is apparent that *T. patula* regulates the amount of DNA in its macronucleus in accordance with the stages of its life history. During vegetative growth the cells more than double their DNA content without undergoing division, while in the reproductive cyst the cells undergo multiple division cycles in the absence of S resulting in a decrease in the DNA content. The basis for this regulation perhaps lies in the fact that *T. patula* (Dysart, 1963), like other ciliates (Raikov, 1968), possesses a highly polyploid macronucleus, and moreover, it is consistent with the idea that the ciliate macronucleus is composed of many independently

segregating genomes (Nanney, 1964). Thus, division without previous DNA synthesis, or unequal macronuclear division, would result in the segregation of genomes to daughter nuclei which would insure that, although the quantity of DNA after division is not the same as that of the parent cell, the genetic information remains unchanged from one generation to the next.

Thus, the present study and others already cited show that the ciliates represent a class of eukaryotic cells in which the replication and segregation of nuclear material is not rigorously coordinated in the cell cycle. The significant question for the future is how the two events are uncoupled.

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