CHANGES IN THE DNA SYNTHESIS PATTERN OF PARAMECIUM WITH INCREASED CLONAL AGE AND INTERFISSION TIME

JOAN SMITH-SONNEBORN and MICHAEL KLASS

From the Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020

ABSTRACT

The clonal age in paramecia refers to the total number of vegetative divisions a clone has undergone since its origin at autogamy (self-fertilization).

As clonal age increases, the interfission time usually increases. The DNA synthesis pattern of cells of different ages was compared by autoradiographic analysis of the DNA synthesis of synchronized cells at various time intervals during the cell cycle (from one division to the next). The study showed that the G_1 period (the lag in DNA synthesis post division) was constant, irrespective of interfission time or clonal age; but the duration of the DNA synthesis period increased with increased interfission time or clonal age. Therefore, we have shown for the first time that the G_1 period is fixed, and the S period is increased in a eukaryotic unicellular organism as a function of interfission time and clonal age.

INTRODUCTION

Paramecium aurelia, a eukaryotic unicellular organism, has been shown to have a well defined life cycle which is characterized by: (*a*) sexual immaturity, when the cells cannot mate or undergo autogamy (self-fertilization); (*b*) maturity when the cells can mate or undergo autogamy; (*c*) senescence, when there is decreased probability that a given cell will give rise to a viable cell at the next division; and (*d*) death, when the cell cannot divide but dies (9, 15). These changes in phenotype occur in the absence of experimentally induced changes in the genotype or environment. Paramecia, therefore, have a limited life span and offer a model system for cellular aging. A limited life span potential has also been found in human diploid cells in culture (4, 5). The paramecium model system of cellular aging offers the unique advantage that the sexual process of mating or autogamy "resets the clock" to zero, reinitiating a new life cycle. Paramecia, like other ciliated protozoans, have two nuclei, the micronuclei and the macronucleus. Only the micronuclear gamete used in fertilization is normally preserved after mating or autogamy, which subsequently generates the new micronuclei and the macronucleus for the progeny cells (3, 14). The micronucleus is considered the germ line, while the macronucleus is considered the somatic line.

Aging cells generally exhibit decreased daily fission rate (15) and therefore presumably increased interfission time (the time from one division to the next). The purpose of the present study was to determine whether the DNA synthesis pattern is constant or variable as clonal age and interfission time increase. Previous studies (1, 2, 6) have found that the macronuclear DNA synthesis pattern in young cells is characterized by: (*a*) a lag period before the onset of DNA synthesis (G_1);

(*b*) a period of DNA synthesis (S); and (*c*) no absence of DNA synthesis (therefore no G_2) before the next division. The present study determines the DNA synthesis pattern of cells of different ages.

Since these cells are large enough to handle easily under a dissecting microscope with a micropipette, synchronously dividing cells were collected and labeled at various intervals during the course of the next generation (1, 2, 6). The cells were then subjected to autoradiographic analysis to detect small amounts of DNA synthesis. Cells of different ages were compared for differences in patterns of DNA synthesis.

MATERIALS AND METHODS

Culture Conditions

Cells of *Paramecium aurelia*, stock 51, sensitive, syngen 4, were kindly supplied by Professor Sonneborn's laboratory, Indiana University. Syngen 4 was used because autogamy can be induced at about 20 fissions after the previous autogamy, and it rarely occurs in daily isolation lines grown in a favorable medium (15). The cells were maintained at 27°C in Cerophyl medium (Cerophyl Laboratories, Kansas City, Kans.) inoculated 24 h before use with *Aerobacter aerogenes* and adjusted to pH 6.7.

Daily Isolation Lines

The procedure of Sonneborn (15) was used for conducting daily isolation lines to obtain aging cells. Our selective procedures differed slightly from those of Sonneborn (15), and are detailed below. Single cells were maintained in plastic disposable spot plates (96 depressions per plate). The spot plates were sterilized with ultraviolet light before use and covered with saran wrap. Autogamy indicated zero time and was routinely ascertained using acridine orange (0.3 mg/ml) staining and fluorescence microscopy to identify the macronuclear changes typical of autogamous cells (21). When 100% of an observed sample of approximately 30 cells exhibited autogamy, eight unstained sister cells were isolated from that depression with a micropipette under a dissecting microscope and removed to a depression containing fresh food as single isolates. The following day the number of cells per depression derived from a single cell was determined, and a single cell from each depression was reisolated and given fresh food. Cells not transferred were permitted to undergo autogamy and served as a source for initiating progeny lines. The procedure of counting cells and reisolation of cells was repeated daily. The $\log_2$ of the number of cells derived from a single cell was the number of fissions per day. The sum of the number of fissions per day from the day of origin at autogamy to a given day was the fission age of that cell. Routinely, eight cells were taken from the clone showing the highest fission rate on the fourth or fifth day postautogamy. This procedure was adopted: (*a*) to minimize the chance that an isolated cell did not undergo autogamy and therefore did not represent a progeny clone; and (*b*) to eliminate those cells from the study which died immediately or within approximately 10–20 fissions after autogamy. (Cells which died immediately or within 10–20 fissions after autogamy were considered by others to be autogamy deaths, not life spans of mitotically dividing cells (7, 15).) Thus, there were eight replicates of one clone. The life span of the isolation line was the sum of the number of fissions from the origin of the clone to the death of the representative of the clone with the longest life span. The procedure is diagrammed below for a given isolation line designated Isolation Line A:

	Sublines							
Day	1	2	3	4	5	6	7	8
1*	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
2	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
3	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
4‡	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
5	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
6	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
7§	0	0	0	0	0	0	0	0
	↓	↓	↓	↓	↓	↓	↓	↓
8	0	0	0	0	0	0	0	0

0 = Depression

* Single isolates in autogamy were placed in fresh food in each of the eight depressions.

‡ All isolates were taken from the depression with the highest fission rate, i.e., subline 4.

§ On day 7 subline 1 was dead, i.e. the cell isolated on day 6 did not survive. A sister cell from subline 2 was used to replace the dead subline. (We usually replace a dead line with a subline to the right of the dead subline.)

Each day the products of a single cell are counted and one cell is reisolated into a new depression in the next row. The back depressions containing the cells which were not transferred were examined for autogamy to initiate a new progeny clone. The replacement procedure of substitution of a dead subline or a subline which undergoes autogamy with a sister subline was used previously, and yields information on the longest possible life span (15). Cells of various ages were used from such isolation lines selected as described above. This procedure generates several isolation lines of cells related as grand-

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020

parent, parent, and daughter, all carried simultaneously. Thus, on any given day, there is an array of cells of different isolation lines available for use in the study.

Occasionally, as mentioned above, a subline would undergo autogamy. This was detected by a decline in the daily fission rate followed by a sharp increase in fission rate during the next 2 days. The occurrence of autogamy was usually confirmed cytologically and the subline was excluded from the isolation line and replaced by a sister subline. Unfortunately, autogamy could not be easily detected in old cells (within 15 fissions of death); the decline in fission rate during or immediately after autogamy could not be distinguished from the decreased fission rate of the old cells. Restoration of the youthful high fission rate does not usually result after autogamy in aged cells (15). Therefore, no data were collected on cells within 15 fissions of the death of the isolation line.

Method of Labeling Cells

The method of Berger and Kimball and Berger (1, 2) was used to label cells. Briefly, *Escherichia coli* T^- (kindly supplied by Dr. James Berger, University of British Columbia, Vancouver, B.C.) was labeled with 50 μCi/ml of tritiated thymidine (specific activity 11 Ci/mM, Schwarz Bio Research Inc., Orangeburg, N.Y.) and was fed to paramecia at the appropriate time for 1-h intervals, or for the entire interfission period. Synchronized cells were obtained by selecting cells with the morphology of dividers and collecting them with a micropipette under a dissecting microscope. Dividing cells were collected over 15-min intervals. At subsequent intervals postdivision, cells were removed from the pooled sample, and incubated at 27°C for 1 h in labeled bacteria. The labeled cells were then removed, washed three times in sterile Cerophyl medium and fixed with 4% neutral formaldehyde. (After fixation cover slips were removed by freezing in liquid nitrogen.) Postfixation was carried out in a 3:1 mixture of absolute ethanol and glacial acetic acid; after this, the slides were passed through 95% ethanol, and finally into 75% ethanol for storage. The slides were hydrated gradually before further processing in aqueous solution.

Enzymatic Treatments

Enzymatic digestions were carried out over 3-h periods at 37°C. Deoxyribonuclease (Worthington, electrophoretically purified) in 4×10^{-3} M $MgSO_4$ and 0.01 M sodium acetate, and ribonuclease (Worthington) were used at a concentration of 0.3 mg/ml. The RNase, in 0.01 M phosphate buffer, was freed of possible DNase activity by heating for 20 min in a boiling water bath. The buffer solutions, free of enzymes, were used as controls. After the respective treatments, the slides were washed in several changes of water, rinsed for 15 min in cold 5% trichloracetic acid, washed again in several changes of water, and partly dehydrated for storage in 75% ethanol.

Autoradiographic Methods

All the slides were covered with Kodak AR-10 stripping film (Eastman Kodak Co., Rochester, N.Y.). They were exposed for approximately 2 wk, developed with Kodak D-19 for 10 min and fixed with standard Kodak fixer.

Preparations were observed using a Zeiss 40x oil-immersion phase-contrast objective (Carl Zeiss, Inc., New York, N.Y.). Grain counts were made using an ocular grid and counting the number of grains per square, in three squares, over the macronucleus. The mean value of the three squares was recorded as the number of grains per unit area in the macronucleus. (Three squares represent about one-third to one-half of the area of the macronucleus observed under our conditions.) This same procedure was used for cells of all ages.

Experimental Design

2 days before a given experiment, all the sublines from different age isolation lines were pooled and grown in mass culture to facilitate obtaining sufficient numbers of dividing cells. A sample of the cells was removed for examination for autogamy; if any cells were found in autogamy, the mass culture was not used. Since variations were found in the incorporation of label from one experiment to the next, only comparisons between different age cells done on the same day are made.

RESULTS

The decline in the mean daily fission rate, typical of aging cells, is seen in the isolation lines used in the present study (Table I). In general, the maximum life span of the clones used was between 175–216 fissions with the exception of clone E, which was a short life span clone. These isolation lines were used as the source of aging cells for the autoradiographic analysis of the DNA synthesis pattern of aging cells.

Evidence for the specificity of the label used for DNA is given in Table II. In confirmation of the previous studies of others (1, 2), the procedure used to label DNA was specific for DNA; 90–95% of the label was removed by electrophoretically purified DNase, but no significant removal was found using RNase or buffer.

Figs. 1–4 show the pattern of DNA synthesis in cells of different ages. Table III summarizes the pattern of DNA synthesis seen in Figs. 1–4. The data indicate that the lag period after division is fixed between 1 and 1.5 h irrespective of the interfission time or clonal age. Therefore, as either interfission time or age increase, the percent of the cell cycle occupied by G_1 decreases.

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020

TABLE I

Mean Daily Fission Rates for Cell Lineages at 5-Day Intervals and Life Span

	Isolation lines							
Day	A	B	C	D	E	F	G	H
5	3.4	4.1	4.5	5.4	4.5	4.3	3.5	3.7
10	4.1	4.8		4.9	4.0	4.7	4.2	4.7
15	4.3	4.5		4.4	4.2	4.9	4.1	4.5
20	5.0	4.4		4.2	3.5	4.7	5.0	4.0
25	4.0	4.1		3.2	2.8	4.4	4.9	4.1
30	4.1	3.9		4.0	0.6	4.0	4.2	3.2
35	3.4	3.7		2.4	0.1	3.1	4.2	3.1
40	2.9	2.9		2.2	0.0	2.3	4.2	3.0
45	2.5	2.8		2.3		2.4	3.1	2.0
50	2.0	2.7		1.2		1.0	2.0	2.0
55	1.8	2.3		1.1		1.0	1.0	1.0
60	0.0	1.8		0.0		0.0	0.0	0.0
65		1.3						
70		0.0						
Maximum life span (fissions)								
	180	216	—	187	100	181	175	180

The mean daily fission rate at 5-day intervals was calculated by averaging the daily fission rate of the eight replicate sublines carried in each isolation line over a 5-day interval of time. Clone C was isolated from a mass culture and was not carried as an isolation line. The clones were related to one another as follows: B was the progeny clone of A, C was a progeny clone of B. E was the progeny clone of D when D was old; G was the progeny clone of F; and H was the progeny clone of G.

Clones D and E were 7 generations removed from A and B. F, G, and H were over 20 generations removed from all other clones. The life spans of the clones were determined as described in the test. With the exception of clone E, all progeny clones were derived from young parents.

TABLE II

Specificity of Label for DNA during One Interfission Period

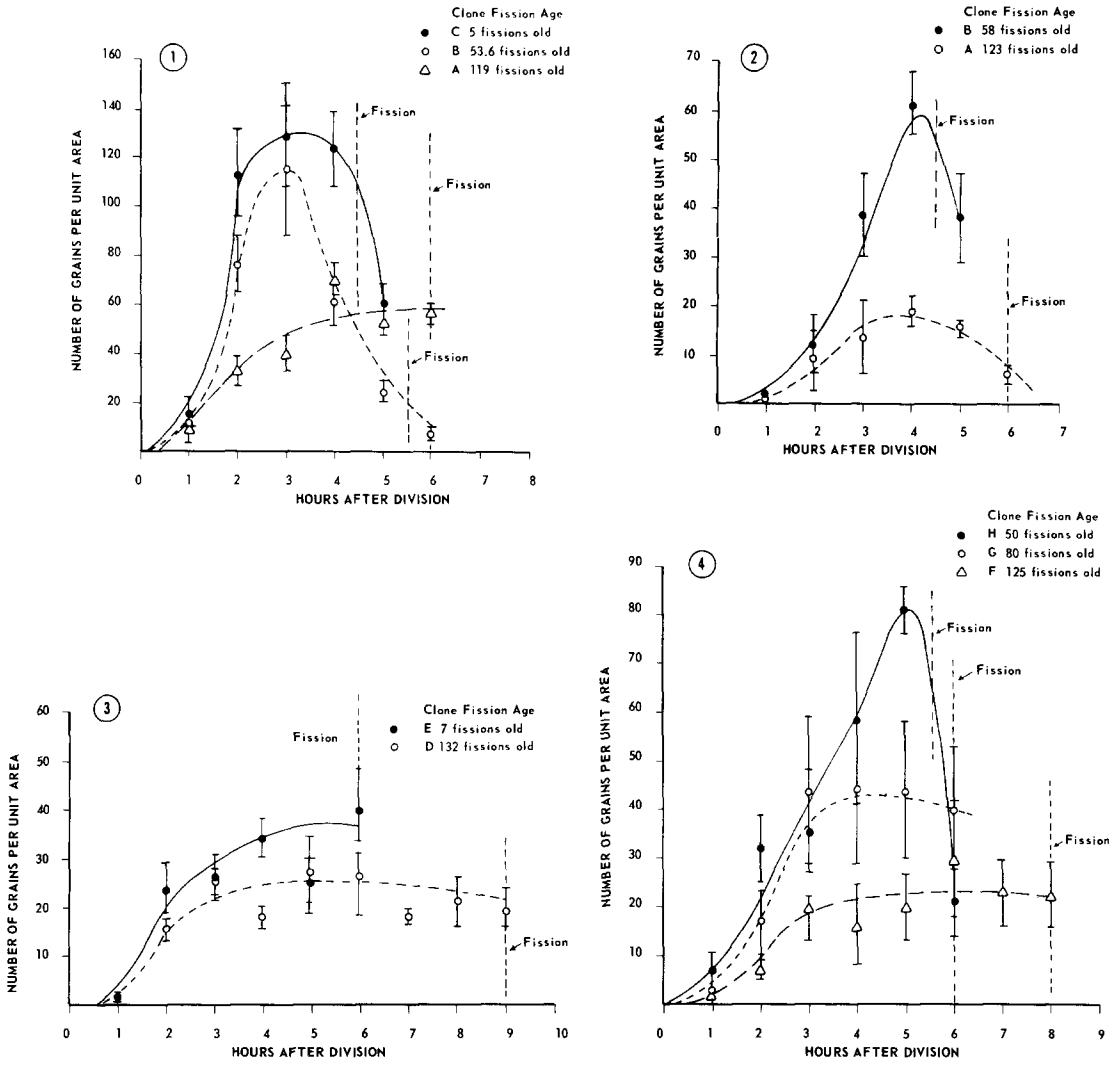
	Treatment			
Age (fissions)	No treatment*	Buffer only*	RNase*	DNase*
150	40.5± 19‡	41.1 ± 6‡	41.2 ± 7.7‡	2.1 ± 1.14‡
50	60.0 ± 10‡	58.2 ± 5‡	61.1 ± 5.2‡	4.1 ± 2.2‡

Synchronously dividing cells were labeled for the entire interfission period. The data indicate that 90–95% of the label is removed by DNase, but not by RNase or buffer. We considered that the removal of grains was essentially complete. The data represent the mean grain counts over five macronuclei and the 95% confidence interval.

* Number of grains per unit area of the macronucleus.

‡ Those means underlined by the same line are not significantly different from one another at the 95% level of confidence, using the Mann Whitney U statistic (13).

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020



FIGURES 1-4 Pattern of macronuclear DNA synthesis. Synchronized cells of different ages were incubated with *E. coli*, T^- labeled with 50 μCi/ml tritiated thymidine for 1-h intervals postdivision. The mean value of 20 macronuclei with the 95% confidence interval for the number of grains per unit area of the macronuclei are recorded: (81 μm^2). The curves were drawn by eye. Each figure represents the values obtained from an experiment done on the same day. The dashed line indicating fission represents the time when the first cell in the population was observed in the next division. 90% of the cells divided within the subsequent 0.5-h interval.

There was no G_2 period (absence of DNA synthesis). The reduced activity per unit area in the macronucleus seen in most young clones just before the next division probably reflects increased nuclear volume (1). This reduced activity per unit area of the macronucleus was not always found in the final period of DNA synthesis. We considered all DNA synthesis post-G_1 as part of the S period.

Within each experiment, as both clonal age and interfission time increased, there was always an increase in the S period. Therefore, both clonal age and interfission period influence the duration

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020

TABLE III

DNA Synthesis Pattern in Aging Cells

Experiment	Clone	Age	Interfission time	Period of DNA Synthesis Cycle	
				G_1	S
			h	h	h
1	C	5	4.5	1.0	3.5
	B	54	5.5	1.0	4.5
	A	119	6.0	1.0	5.0
2	B	58	4.5	1.5	3.5
	A	123	6.0	1.5	4.5
3	E	7	6.0	1.5	4.5
	D	132	9.0	1.5	7.5
4	H	50	5.5	1.5	4.0
	G	80	6.0	1.5	4.5
	F	125	8.0	1.5	6.5

In the four experiments above, cells of different fission ages were fed tritiated thymidine labeled *E. coli* (50 μCi/ml) for 1-h intervals. The cells were processed for autoradiographic analysis as described in the text. The mean number of grain counts were plotted in Figs. 1–4. The G_1 and S periods were taken from Figs. 1–4 and are defined as follows: G_1 was the lag period in incorporation (in hours) immediately after division; the S period was the entire period (in hours) post G_1 to the beginning of the next division.

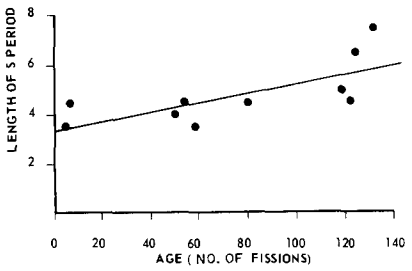


FIGURE 5 Relation of age and DNA synthesis period. The data from Table III were used to compare clonal age and duration of the S period, irrespective of interfission time. The estimated regression time is S = 3.38 + (.018) age where 3.38 is the y intercept (S), and .018 is the slope. The correlation coefficient was .70 which is different from zero at the .05 level.

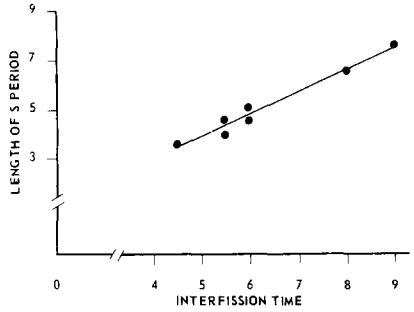


FIGURE 6 Relation of interfission time and DNA synthesis period. The data from Table III were used to compare interfission time and duration of the S period, irrespective of clonal age. The estimated regression line is S = −0.63 + (.89) interfission time where −0.63 is the y intercept (S) and .89 the slope. The correlation coefficient was .98 which is significantly different from zero.

of the S period. If all experiments are considered for the comparison of clonal age and duration of the S period, irrespective of interfission time, the correlation coefficient between the duration of S and clonal age is .70, which is significantly different from zero at the .05 level. The estimated regression line is S = 3.38 + (.018) clonal age (Fig. 5). Likewise, if duration of the S period and interfission time are compared, irrespective of clonal age, the correlation coefficient is .98, and the re-

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020

gression line is S = −.63 + (.89) interfission time (Fig. 6). These calculations serve to strengthen the conclusion that clonal age and interfission time influence the duration of the S period, as seen in the experiments done on the same day.

It is, of course, possible that the differences between experiments contribute to the correlations found, i.e. differences in available food on different days may contribute to differences in S periods. Therefore, the data within the individual experiments showing the correlation of increased interfission time and increased clonal age with duration of the S period are the only data considered as proof of the above relationship.

Examination of Figs. 1–4 also reveals that the maximum activity per unit area of the macronucleus was always less in older cells in each experiment. There are eight such comparisons of younger versus older cells. The probability of this happening by chance alone, if there was no difference between younger and older clones in the observed population, is 0.004. It is very unlikely, therefore, that the observed difference in the maximum activity per unit area of the macronucleus was due to chance and is considered a difference between young and old cells. The present data do not permit conclusions on the basis of the difference in the maximum activity per unit area in the macronucleus in young and old cells.

DISCUSSION

The data show that, in paramecia, the pattern of DNA synthesis is not fixed but can vary with interfission time and clonal age. Our results confirm the pattern of DNA synthesis found in young cells by Berger (1) and extend his studies to include the DNA synthesis pattern of aging cells. For the first time, it has been shown that: (*a*) the G_1 period of macronuclear DNA synthesis cycle is of constant length; thus as the interfission time and clonal age increase, the percent of the cell cycle occupied by G_1 decreases; (*b*) the period of macronuclear DNA synthesis increases with increased interfission time and clonal age, and usually at the expense of the decline in DNA synthesis found in the final period of the cell cycle before the next division. The S period increased from 3.5–7.5 h as clonal age increased from 5–132 fissions. One young clone (clone E, 7 fissions old) exhibited a long interfission period and a corresponding longer S period of 4.5 h. Clone E was the only known short life span clone used in the present study. Clone E was initiated from clone D when clone D was aged. Recent studies in our laboratory indicate that as parental age increases, progeny life span decreases (11). Although one exceptional clone is not sufficient evidence to assume that parental age may be a variable in the DNA synthesis of progeny clones, it does serve as an indicator that parental age should be tested as a possible variable in the DNA synthesis pattern of progeny cells. Siegel (9) found that increased parental age reduced the immaturity period (the number of fissions between origin at autogamy and the capacity to mate) in progeny cells. Both Siegel's study and our recent study show that parental age can be a variable in the determination of progeny phenotype. Also, these studies indicate that the fission age alone is not an absolute indicator of the vigor or stage in the life cycle of the clone.

Since the time occupied by DNA synthesis increased with increased clonal age, then either the rate of DNA synthesis is reduced in older cells, more time is required for DNA repair, or the resulting cells will have more DNA. It is likely that the older cells may require more time for DNA repair. Sonneborn and Schneller (16) found that micronuclear mutations increased with clonal age, and it is reasonable to assume that there may also be macronuclear mutations in older cells, though no method has yet been developed to detect them. Further, it is known that sensitivity to ultraviolet light increases with advancing clonal age, suggesting that old cells have a reduced capacity for DNA repair (10). Therefore, it would seem likely that the old cells would need more time for repair of DNA during the longer S time. The present study was not designed to determine either the total rate of DNA synthesis or the total amount of DNA in old and young cells; such determinations require estimates of the relative changes in nuclear volume and mass during the cell cycle or microspectrophotometry in Feulgen-stained cells. Nevertheless, in all experiments the maximum number of grains per unit area was always lower for older cells. Possible mechanisms for the reduced activity per unit area in older cells are: (*a*) increased nuclear volume or mass; (*b*) increased soluble DNA precursor pools; (*c*) decreased permeability of the macronucleus to DNA precursors; (*d*) decreased efficiency of the enzymes required for DNA replication; (*e*) decreased ability to ingest bacteria or form food vacuoles; and (*f*) less total DNA. A short note by Schwartz and Meister appeared re-

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020

cently showing that the DNA in *Paramecium* macronuclei decreases as fission age increases (8). Their conclusion was based on scanning microspectrophotometric analysis of Feulgen-stained aging cells. Less DNA in the macronucleus, then, could contribute or even account for the reduced activity per unit area in the macronucleus of older cells, reported in this paper. Their study (8) and our supportive data could provide a physical basis for a possible mechanism for age-correlated changes in phenotype, ie. some loss of copies of genes in the polyploid nucleus. Other experiments can be performed to ascertain the contribution of some or all of the possible variables which could result in the observed reduced number of grains per unit area in the macronucleus of the older cells.

The contribution of the present study, then, is that the DNA synthesis pattern is not fixed, but can vary with increased clonal age and interfission time. Aging mechanisms do not interfere with the absolute time for the G_1 period within the age intervals investigated. We have shown, for the first time, that the G_1 period is constant, irrespective of age and interfission time. As the interfission time and clonal age increase, the remainder of the cell cycle is used for DNA synthesis.

We would like to express our thanks to Dr. Lyman McDonald for his assistance in statistical methods, and to Daniel Cotton for technical assistance.

This study was supported in part by grants from the National Science Foundation (GB31 623); and the Arts and Sciences Basic Research Fund, and Graduate School, University of Wyoming.

Received for publication 26 July 1973, and in revised form 10 December 1973.

REFERENCES

1. BERGER, J. 1971. Kinetics of incorporation of DNA precursors from ingested bacteria into macronuclear DNA of *Paramecium aurelia*. *J. Protozool.* **18**:419.
2. BERGER, J. D., and R. F. KIMBALL. 1964. Specific incorporation of precursors into DNA by feeding labeled bacteria to *Paramecium aurelia*. *J. Protozool.* **110**:534.
3. DILLER, W. F. 1936. Nuclear reorganization processes in *P. aurelia* with descriptions of autogamy and 'hemixis'. *J. Morphol.* **59**:11.
4. HAYFLICK, L. 1965. The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* **37**:614.
5. HAYFLICK, L. 1970. Aging under glass. *Exp. Geront.* **5**:291.
6. KIMBALL, R. F., and S. W. PERDUE. 1962. Quatitative cytochemical studies on *Paramecium aurelia*. V. Autoradiographic studies of nucleic acid synthesis. *Exp. Cell Res.* **27**:405.
7. PIERSON, B. F. 1938. The relation of mortality after endomixis to the prior interendomictic interval on *Paramecium aurelia*. *Biol. Bull. (Woods Hole)*. **24**:238.
8. SCHWARTZ, V., and H. MEISTER. 1973. Eine Altersveranderung des Makronucleus von *Paramecium*. *Z. Naturforsch. Teil B.* 28c, Heft 3/**4**:232.
9. SIEGEL, R. W. 1961. Nuclear differentiation and transitional cellular phenotypes in the life cycle of *Paramecium*. *Exp. Cell Res.* **24**:6.
10. SMITH-SONNEBORN, J. 1971. Age correlated sensitivity to ultraviolet radiation in *Paramecium*. *Radiat. Res.* **46**:64.
11. SMITH-SONNEBORN, J., M. KLASS, and D. COTTON. 1974. Parental age and life span versus progeny life span in *Paramecium*. *J. Cell Sci.* **14**:1.
12. SMITH-SONNEBORN, J. 1974. Acridine orange fluorescence: a temporary stain for *Paramecium*. *Stain Technol.* In press.
13. SOKAL, R. R., and J. F. ROHLF. 1969. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Co., San Francisco. 392, 402.
14. SONNEBORN, T. M. 1939. Genetic evidence of autogamy in *P. aurelia*. *Anat. Res.* **75**:85.
15. SONNEBORN, T. M. 1954. The relation of autogamy to senescence and rejuvenescence in *Paramecium aurelia*. *J. Protozool.* **1**:38.
16. SONNEBORN, T. M., and M. SCHNELLER. 1960. Age induced mutations in *Paramecium*. *In* Biology of Aging. B. L. Strehler, editor. Waverly Press, Baltimore, Maryland. 286.

Downloaded from http://rupress.org/jcb/article-pdf/61/3/591/1071032/591.pdf by guest on 03 December 2020