

# REGULATION OF MACRONUCLEAR DNA CONTENT IN *PARAMECIUM TETRAURELIA*

JAMES D. BERGER and HELMUT J. SCHMIDT

From the Department of Zoology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5. Mr. Schmidt's present address is Zoologisches Institut der Westfälischen Wilhelms-Universität, Münster, W. Germany.

## ABSTRACT

The macronucleus of *Paramecium* divides amitotically, and daughter macronuclei with different DNA contents are frequently produced. If no regulatory mechanism were present, the variance of macronuclear DNA content would increase continuously. Analysis of variance within cell lines shows that macronuclear DNA content is regulated so that a constant variance is maintained from one cell generation to the next. Variation in macronuclear DNA content is removed from the cell population by the regulatory mechanism at the same rate at which it is introduced through inequality of macronuclear division. Half of the variation in macronuclear DNA content introduced into the population at a particular fission by inequality of division is compensated for during the subsequent period of DNA synthesis. Half of the remaining variation is removed during each subsequent cell cycle. The amount of variation removed in one cell cycle is proportional to the postfission variation. The cell's power to regulate DNA content is substantially greater than that required to compensate for the small differences that arise during division of wild-type cells. For example, a constant variance was still maintained when the mean difference between sister cells was increased to ten times its normal level in a mutant strain.

The observations are consistent with a replication model that assumes that each cell synthesizes an approximately constant amount of DNA which is independent of the initial DNA content of the macronucleus. It is suggested that the amount of DNA synthesized may be largely determined by the mass of the cell.

**KEY WORDS** *Paramecium tetraurelia* · DNA synthesis · regulation of DNA content · variances of DNA content · cell cycle

The ciliate macronucleus divides amitotically with neither chromosome condensation nor the formation of a typical mitotic spindle. In the absence of mitosis or an equivalent process, equal distribu-

tion of macronuclear DNA does not necessarily occur, and daughter cells with unequal DNA contents are frequently produced (5, 9, 12-15, 18, 19, 27). This inexact distribution of DNA to daughter macronuclei is presumably tolerated because the polygenomic macronucleus (1, 7, 26) contains a large number of redundant functional subunits (8, 21). Although each subunit presum-

ably replicates completely, these units may be partitioned unequally to the daughter macronuclei at cell division. Nevertheless, ciliates would be expected to have evolved a mechanism to compensate for variation in macronuclear DNA content so that a constant mean DNA content with a constant variance would be maintained over many cell generations. If a regulatory mechanism were absent, the variance of macronuclear DNA content within the population would increase continuously.

The presence of such a mechanism in *Paramecium* has been inferred by Kimball (13) from two observations. First, an approximately constant variance in macronuclear DNA content was maintained over many cell cycles. Second, the coefficient of variation of macronuclear DNA content in postreplication cells was less than that in pre-replication cells. This indicated that regulation may occur during the period of macronuclear DNA synthesis which takes place during the last three-quarters of the cell cycle (2). He also suggested that regulation of DNA content was a slow process that extended over several cell generations (13). Regulation of DNA content in *Paramecium* cannot occur at fission as it does in *Tetrahymena* strains which produce chromatin extrusion bodies (5), for all of the parental DNA is distributed to the daughter cells (14).

The present study examines the ability of *Paramecium* to regulate its macronuclear DNA content, the kinetics of regulation, and the nature of the compensatory process. The variability of macronuclear DNA content was increased through the action of gene mutations so that the extent of the cell's regulative ability could be determined. The innovation of this study is the application of the hierarchical or nested analysis of variance technique (20) to the problem. This allows the separation and removal of variance arising before the start of the experiment, and makes it possible to follow variation introduced at a particular fission through subsequent replication events without interference from preexistent or subsequently introduced variation.

## MATERIALS AND METHODS

### *Stocks and Culture of Paramecium*

*Paramecium tetraurelia* (24) stock 51-S (wild type), and two derived stocks, d4-43 and d4-1030, carrying mutant genes, were grown in grass infusion at 27°C

(23). The food organism was *Klebsiella pneumoniae*. Stock d4-43 carries the recessive mutation *am* (22) which causes partial or complete missegregation of macronuclei to daughter cells in a variable fraction of the cells, and stock d4-1030 carries a new mutation *tam-A* (16) which causes both macronuclear missegregation and absence of trichocyst discharge: the penetrance and expressivity of *tam-A* is much greater than that of *am*; the phenotype is similar to that of *tam-38* (17), but *tam A* and *tam-38* are not allelic (16).

### *Cytochemical Procedures*

Newly divided cells were individually transferred to an albumin-coated microscope slide by micropipette, and as much culture medium as possible was withdrawn so that maximum flattening occurred as each cell dried.

After drying, cells were fixed in ethanol, acetic acid (3:1) mixture for 20 min, rinsed in water, hydrolyzed for 15–20 min in 3.5 N HCl at 37°C (10), and stained by the Feulgen procedure using freshly prepared stain. After staining, the slides were rinsed in acid-bisulfite solution, then in running water for an hour, and allowed to air dry.

The locations of the groups of cells on each slide were marked with drawing ink to facilitate microscope observation. The cells were mounted in Cargille's refractive index oil ( $n = 1.534$ , R. P. Cargille Labs, Inc., Cedar Grove, N. J.) under number 1 coverslips which were sealed with fingernail polish.

Absorption microspectrophotometric measurements of the Feulgen's-stained macronuclei were made at 570 nm with a Zeiss microspectrophotometer equipped with a scanning stage (0.5- $\mu$ m step). The macronuclei were scanned in a rectangular array of equidistant points, and the encoded intensity data were stored on paper tape. The paper tape records were processed by computer to produce a two-dimensional array of absorbance measurements. Absorbance due to extraneous absorbing materials (food vacuoles, other nuclei, or debris) was subtracted to produce a corrected cumulative extinction value for each nucleus which was assumed to be proportional to the amount of dye bound by the object. Instrument error and processing error together were < 2.5% throughout the experiments.

### *Statistical Procedures*

The DNA content of pre-fission (postreplication) cells was estimated by adding the DNA content of the two newly divided daughter cells as justified by Kimball and Barka (14). The difference between the postreplication DNA content of sister cells was estimated by the differences between the sums of the DNA contents of their daughter cells. The variation of DNA content in samples was measured by the coefficient of variation ( $100SD/\bar{x}$ ). Other statistical procedures were carried out as de-

scribed by Sokal and Rohlf (20). Means are shown with their standard errors.

### Computer Simulation of Model II Replication

A computer simulation routine was used to explore the consequences of varying the value of the threshold parameter,  $t$ , of the model II replication function (1) on the resulting DNA content variances. An initial set of 1,000 normally distributed prereplication DNA contents with mean and standard deviation equal to the observed values for wild-type *Paramecium* cells was generated. From each prereplication value a corresponding postreplication value was generated by application of the replication function (Eq. 1). The prereplication values for the next generation were generated by application of the division function (Eq. 2) to each postreplication value. The replication and division processes were repeated for each generation. The replication function was:

$$x_2 = \begin{cases} x_1 & \text{if } x_1 > t_u \\ 2x_1 & \text{if } t_u \geq x_1 \geq t_l \\ 4x_1 & \text{if } x_1 < t_l \end{cases} \quad \text{when } t_u = \bar{x}(t) \text{ and } t_l = \bar{x}/t \quad (1)$$

where  $x_1$  is the prereplication value,  $x_2$  is the postreplication value,  $\bar{x}$  is the mean prereplication value,  $t_u$  and  $t_l$  are the upper and lower threshold values, respectively, and  $t$  is the threshold parameter. The division function was:

$$x_1 = (x_2 \pm d)/2 \quad (2)$$

where  $d$  is a normally distributed random variable with mean and standard deviation equal to the observed values for differences between wild-type sister cell DNA contents. The sign of  $d$  was assigned at random to each value.

### RESULTS

In each of several experiments, a number of cell lines was initiated by selecting dividing cells from a young exponential-growth-phase culture. These founder cells were allowed to grow and to divide two or three times. The newly divided progeny cells were then fixed, and the macronuclear DNA content of each cell was estimated by microspectrophotometry. Within each cell line the relations of the cells to each other by descent from the founder cell was known. The design of the experiment is shown in Fig. 1. Three sets of data were generated, one for each of the three genotypes used (wild-type, *am/am*, and *tam A/tam A*). These data sets provided the basis for all subsequent observations and analyses.

### Distribution of Macronuclear DNA to Daughter Cells

The difference in macronuclear DNA content between sister cells of each of the three genotypes was expressed as a fraction of the parental prereplicative DNA content (Fig. 2). In wild-type cells the mean difference between sister macronuclei was  $2.9 \pm 0.4\%$  of the parental DNA content. In *am/am* cells the difference increased to  $8.4 \pm 1.1\%$ , and nearly half of the cells showed greater inequality of division of macronuclear DNA than did any of the wild-type cells. However, in *tam A/tam A* cells, missegregation of macronuclear DNA was much more severe and the average difference between sister macronuclei was  $31.5 \pm 0.5\%$  of the parental DNA content. Approximately 15%

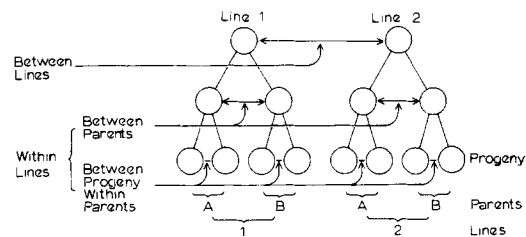


FIGURE 1 Design of "pedigree experiment" for study of variation in macronuclear DNA content within cell lines by nested analysis of variance.

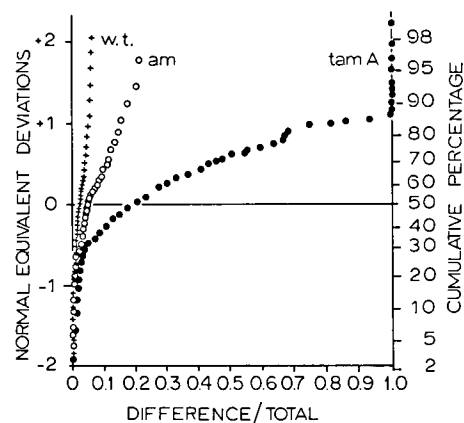


FIGURE 2 Normalized cumulative distributions of the inequality of macronuclear division in different genotypes. The degree of inequality of macronuclear division is expressed as the difference between the DNA contents of postfission sister cells as a fraction of the parental DNA content. (+) Wild-type (w.t.) cells,  $n = 159$ ; (o) *am/am* cells,  $n = 113$ ; and (●) *tam A/tam A* cells,  $n = 120$ .

of the cells showed complete missegregation of the macronucleus so that the entire parental macronucleus passed intact to one of the daughter cells, and the other daughter cell received no macronucleus at all. Expression of the mutant phenotype was quite variable. Although half of the sample showed inequalities of macronuclear division greater than those in any of the *am/am* cells, more than 30% of the cells showed almost equal division with inequalities within the wild-type range.

The substantial inequality of the distribution of macronuclear DNA to daughter cells in the mutant strains made it possible to test the organism's power to regulate macronuclear DNA content.

### Regulation of DNA Content

The question of the occurrence, extent, and overall kinetics of regulation of macronuclear DNA content was approached through analysis of variance of macronuclear DNA content within cell lines. In each experiment the total variance in macronuclear DNA content can be separated into two major components: that occurring between cell lines and that occurring within cell lines. The between-lines variance component was assumed to have arisen before the start of the experiment and was discarded. The within-lines component contains the variance in macronuclear DNA content that arose during the experiment. This variance consists, in turn, of at least two components, for variance in macronuclear DNA content is introduced at each fission through inequalities in

macronuclear division. The variance between the macronuclear DNA contents of sister cells is an estimate of the variance introduced at each fission. The variance between the parental (postreplication) DNA contents (estimated by the sum of the DNA contents of the daughter cells) allows estimation of the variance remaining after completion of one period of DNA synthesis. In the same way, variance between sublines in a three-generation experiment provides an estimate of the variance left after two periods of DNA replication.

If regulation of DNA content during the period of DNA synthesis did not occur, the variance of the postreplication DNA content would be expected to be four times larger than that in prereplication cells (Table I). Over several generations the variance would be expected to increase continuously as new variance would be added at each fission through inequalities of macronuclear DNA distribution to daughter cells without removal of any of the existing variance. The coefficients of variation of macronuclear DNA content would also be expected to be the same in both pre- and postreplication cells (Table I). If, on the other hand, regulation of macronuclear DNA content were occurring during the period of DNA synthesis, the overall variance in macronuclear DNA content would remain constant, and old, preexisting variance would have to be removed as rapidly as new variance was introduced by inequalities of macronuclear division. Finally, if perfect regulation of DNA content occurred, the coefficient of variation (standard deviation as a percentage of the mean) of the macronuclear DNA content in

TABLE I  
Properties of Populations with and without Regulation of DNA Content during the Period of DNA Synthesis

Without regulation	With regulation
<b>Assumptions</b>	
1. Exact doubling of DNA in each cell during each cycle	1. Mean DNA content of the population doubles during each cell cycle 2. Total variance of DNA content is constant
<b>Consequences</b>	
1. $\bar{x}_2 = 2\bar{x}_1$	1. $\bar{x}_2 = 2\bar{x}_1$
2. $s_2^2 = \sum(2x_1 - 2\bar{x}_1)^2/n - 1 = 4s_1^2$	2. $s_2^2 = s_1^2$
3. $s_2 = 2s_1$	3. $s_2 = s_1$
4. $cv_2 = 100(s_2/\bar{x}_2) = 100(2s_1/2\bar{x}_1) = cv_1$	4. $cv_2 = 100(s_2/\bar{x}_2) = 100(s_1/2\bar{x}_1) = 1/2 cv_1$
<b>Where</b>	
$\bar{x}_1$ = mean prereplication DNA content;	$s_1$ = its standard deviation; $s_1^2$ = its variance; $cv_1$ = its coefficient of variation;
$\bar{x}_2$ = mean postreplication DNA content;	$s_2$ = its standard deviation; $s_2^2$ = its variance; $cv_2$ = its coefficient of variation

postreplication cells should be half of that in prereplication cells (Table I).

Analysis of variance within cell lines shows that regulation of macronuclear DNA content occurs in all three genotypes (Table II). In all genotypes, the variance of the parent (postreplication) cells was equal to or less than that of the progeny (prereplication) cells, as shown by a 1-tailed F test (Table III). Consequently, there is a reduction by at least one-half of the coefficient of variation between progeny and parent cells (Table II). A semilogarithmic plot of the coefficients of variation reveals that regulation of DNA content occurred over two successive generations (*am/am* data) and that the fractional reduction of the

coefficient of variation is largely independent of the magnitude of the variation in sister cell DNA content. Conversely, the absolute magnitude of the reduction of the variation per generation increases as the total variation increases (Fig. 3).

The greater than twofold reduction in the coefficient of variation in the *tam A/tam A* experiments (Table II) is probably an artifact because several cell lines were lost from each experiment through complete missegregation of the macronucleus to one daughter cell at the first fission after the start of the experiment. Thus, the lines making the greatest contribution to the variance of the parental generation were lost. There was an approximate halving in the coefficient of variation

TABLE II  
Nested Analysis of Variance Tables for Pedigree Experiments

Experiment	Variance component	df*	variance(MS)‡	Total vari-	Within lines	CV ± s <sub>cv</sub> §
				ance	variance	
				%	%	
<b>JB-1</b>						
Wild-type four-cell pedigrees	Total	107	174.2	100		
	Between lines	26	83.9	48		3.2 ± 0.4
	Within lines	81	90.3	52	100	
	Between parents	27	37.7	22	42	4.3 ± 0.6
	Between progeny	54	52.6	30	48	10.1 ± 1.0
<b>HS-1</b>						
<i>am/am</i> four-cell pedigrees	Total	111	1,269.2	100		
	Between lines	27	973.3	77		14.6 ± 2.0
	Within lines	84	295.9	23	100	
	Between parents	28	149.6	12	50	11.4 ± 1.5
	Between progeny	56	146.4	12	50	22.7 ± 2.3
<b>HS-2</b>						
<i>am/am</i> eight-cell pedigrees	Total	103	1,296.7	100		
	Between lines	12	872.9	67		7.1 ± 1.5
	Within lines	91	423.7	33	100	
	Between sublines	13	105.9	8	25	4.9 ± 0.9
	Between parents	26	160.8	12	38	12.2 ± 1.7
	Between progeny	52	157.0	12	37	24.0 ± 2.5
<b>JB-2</b>						
<i>tam A/tam A</i> four-cell pedigrees experiment 1	Total	171	5,009.5	100		
	Between lines	42	790.1	16		7.3 ± 0.8
	Within lines	129	4,219.4	84	100	
	Between parents	43	1,180.2	24	29	19.1 ± 2.1
	Between progeny	86	3,139.2	61	72	61.6 ± 6.2
<b>HS-3</b>						
<i>tam A/tam A</i> four-cell pedigrees experiment 2	Total	67	15,245	100		
	Between lines	16	3,316	22		13.7 ± 2.5
	Within lines	51	11,885	78	100	
	Between parents	17	3,810	25	32	29.2 ± 5.4
	Between progeny	34	8,076	53	68	85.4 ± 16.2

\* Degrees of freedom.

‡ Mean square.

§ Standard error of the coefficient of variation.

in both *am/am* and wild-type cell lines in which complete missegregation did not occur (Fig. 3).

Although all three genotypes with differing degrees of inequality of distribution of macronuclear DNA to daughter macronuclei maintained a constant variance in macronuclear DNA content from one generation to the next, there was a proportional increase in the coefficient of variation of the parental (postreplication) DNA content as the inequality of division increased (Table IV).

TABLE III  
Ratio of Parent Variance to Progeny Variance in Four-Cell Pedigree Experiments

Genotype	F (parents/progeny)	df	Probability of larger F
Wild-type	0.717	27,54	0.82
<i>am/am</i>	1.002	28,56	0.46
<i>tam A/tam A</i>	0.377	43,86	0.99

$H_0: s_1^2 < s_2^2$ . Legend as in Table I.

#### How Does Regulation Occur?

Regulation of macronuclear DNA content during the period of DNA synthesis could occur in several ways. Two models for the control of the amount of DNA synthesized in individual cells during a single interfission interval are presented below and considered further in the Discussion section. Model I (developed from the present data) permits partial "rounds" of DNA synthesis in macronuclei. The amount of DNA synthesized is the same in all cells and is independent of the

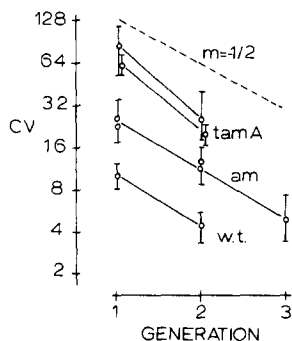


FIGURE 3 Semilogarithmic plot of the coefficients of variation of macronuclear DNA content after successive rounds of DNA synthesis. The uppermost line shows the slope expected for a halving of the coefficient of variation with each round of DNA synthesis, as would be expected if regulation of macronuclear DNA content occurred during the period of DNA synthesis.

TABLE IV  
Relation between Inequality of Division and Variability of Prefission DNA Content

Genotype	Mean inequality of macronuclear division as % of prefission DNA	Coefficient of variation of prefission DNA
+/+	5.0 ± 0.6	4.3 ± 0.6
<i>am/am</i>	8.2 ± 1.0	11.4 ± 1.5
<i>tam A/tam A</i>	31.1 ± 4.0	29.2 ± 5.4

prereplication DNA content of the macronucleus. Model II (developed by Doerder and DeBault [9] for *Tetrahymena*) requires that complete rounds of macronuclear DNA synthesis occur. The number of rounds of synthesis that occur is determined by the prereplication DNA content of the macronucleus.

The models were tested by comparison of observed sets of postreplication DNA content values with sets of expected postreplication values generated by the models. The observed sets of postreplication DNA content values were obtained by summing the DNA contents of prereplication sister cells. The sets of expected postreplication DNA content values were obtained by applying the appropriate replication function to the observed sets of prereplication DNA content values. The replication function for model I was:

$$x_2 = x_1 + \bar{x}_1,$$

and for model II was:

$$x_2 = \begin{cases} x_1 & \text{if } x_1 > t_u \\ 2x_1 & \text{if } t_u \geq x_1 \geq t_l \\ 4x_1 & \text{if } x_1 < t_l \end{cases}$$

when:  $t_u = \bar{x}_1(t)$   
and  $t_l = \bar{x}_1/t$

where  $x_2$  was the estimated postreplication DNA content,  $x_1$  was the observed prereplication DNA content,  $\bar{x}_1$  was the observed prereplication mean DNA content, and  $t_u$  and  $t_l$  were the upper and lower threshold values, respectively. The threshold parameter,  $t$ , was set equal to  $\sqrt{2}$ . The value chosen was based on observations of Cleffmann (5) and Doerder and DeBault (unpublished data) for *Tetrahymena*. The value is not arbitrary, for a computer simulation of model II replication reveals that a shift of the threshold parameter in either direction results in a dramatic increase in

the coefficient of variation of the DNA content (Fig. 4).

If the models produced adequate regulation of DNA content, the means and variances of the sets of expected postreplication DNA contents should be similar to the observed values. This was the case when either model was applied to the highly variable *tam A/tam A* data (Table V). However, when model II was applied to the much less variable wild-type data, the predicted postreplication variance was approximately twice the observed value.

The two replication models differ fundamen-

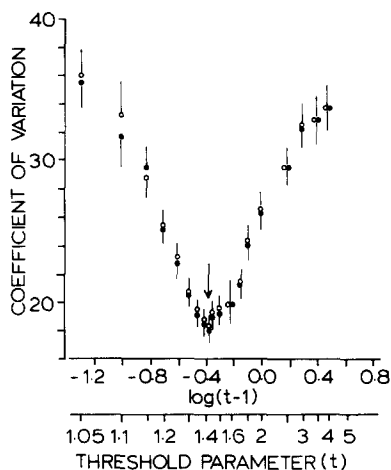


FIGURE 4 Effect of variation of the threshold parameter,  $t$ , of the replication function for model II on the predicted coefficients of variation of the DNA content of prereplication (O) and postreplication (●) cells. The values shown were obtained at the 100th generation of simulated replication and division. Sample size equals 1,000 cell lines. The  $t$  values are plotted on a logarithmic scale. The vertical arrow indicates the value of  $t$  chosen for application of model II to the experimental data. Vertical bars are 95% confidence limits of the coefficient of variation.

tally in the nature of the replication process. Model I is additive, whereas model II is multiplicative. The most precise test of the nature of the replication process in *Paramecium* involves comparison of the differences between sister cell DNA contents before and after replication. Prereplication differences were obtained directly from the progeny cell data sets. Postreplication differences were obtained by summing the DNA contents of the daughter cells produced by fission of each of the parent sister cells (designated A and B in Fig. 1). These data were compared with sets of expected postreplication sister cell differences obtained by applying the appropriate replication functions to the prereplication sister cell DNA content values. The data sets were ranked and the cumulative distributions were plotted. The distribution of prereplication sister cell differences and the expected distribution of postreplication differences generated by model I are the same; model I does not change the difference between sister cell DNA contents because all cells synthesize the same amount of DNA. However, model II, when applied to the wild-type data, predicted that the differences between sister cell DNA contents should double during the replication process. The observed distribution of postreplication differences was closer to the expectation of model I than it was to that of model II (Fig. 5). Plotting the differences between observed pre- and postreplication sister cell differences of the same percentile rank in their distributions as a function of the magnitude of the prereplication difference revealed that there was an approximately constant increase in the sister cell difference during replication. This was inconsistent with the expectation for model II and was much closer to the expectation for model I (Fig. 6). This result showed unequivocally that DNA replication in wild-type *Paramecium* cells was essentially an additive proc-

TABLE V  
Comparison of Observed Variances of Postreplication DNA Content with Those Generated by Specific Replication Models

Genotype	Observed values		Expected values					
	df*	Variance	Model I			Model II		
			df*	Variance	P‡	df*	Variance	P‡
Wild-type	55	105.1	111	59.1	0.005	111	207.6	0.005
<i>tam A/tam A</i>	77	1,954	149	1,563	0.1	149	1,674	0.1

\* Degrees of freedom.

‡ Probability of homogeneity with observed value by F test.

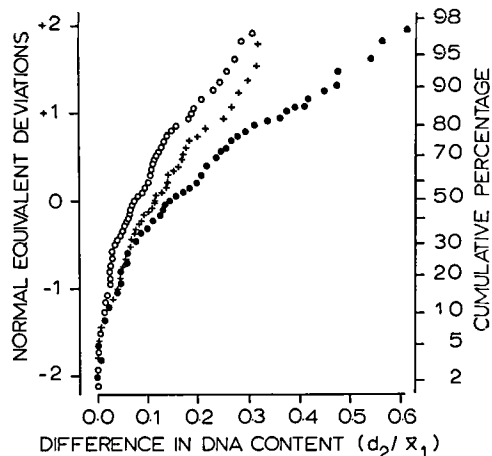


FIGURE 5 Normalized cumulative distributions of differences between postreplication sister cell DNA contents in wild-type cells. (+) Observed differences in DNA content,  $n = 39$ ; (O) result expected from model I,  $n = 56$ ; (●) result expected from model II,  $n = 56$ . Differences in DNA content are expressed as fractions of the mean prereplication DNA content.

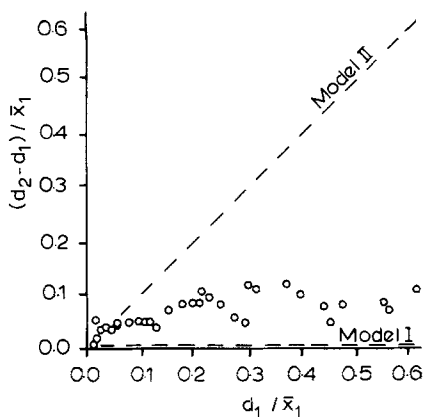


FIGURE 6 Increase in the difference between sister cell DNA contents of the same percentile rank during replication as a function of the initial magnitude of the differences. All values are expressed as fractions of the mean prereplication DNA content ( $\bar{x}_1$ ).  $d_1$  and  $d_2$  are the differences between sister cell DNA contents before and after replication, respectively;  $n = 28$ . The expectations of model I and model II are indicated by the dashed lines.

ess rather than a multiplicative process as implied by model II. If replication were multiplicative, the increases in the sister cell difference that occurred during replication should have become greater as the prereplication difference increased.

## DISCUSSION

*Paramecium* possesses far greater ability to regulate macronuclear DNA content than is required to compensate for the relatively small differences in macronuclear DNA content that occur between sister cells in wild-type organisms. The cells maintain a constant variance in macronuclear DNA content even when the mean difference between sister cell DNA content is increased to ten times the wild-type level by the action of mutant genes.

This study confirms the occurrence of regulation of macronuclear content and reveals its overall dynamics. In each cell cycle, half of the total variation in macronuclear DNA content present at the start of the cell cycle is removed. When the population is at equilibrium, the rate at which the regulatory mechanism removes variation during DNA replication balances the rate at which new variation is introduced through inequalities in the distribution of DNA to daughter macronuclei. Thus, a constant variance in macronuclear DNA content is maintained by all three genotypes. However, the absolute magnitude of the variation of the postreplication DNA content (the unregulated remainder of the variation introduced at the previous fission) increases with the degree of inequality of the distribution of DNA to sister macronuclei.

The variation in macronuclear DNA introduced at a particular fission is gradually eliminated from the population over the course of a number of cell generations. In each successive generation, half of the remaining variation is removed. Progressively smaller fractions of the original variation are removed in each successive cell cycle. The magnitude of the variation removed, however, is not fixed, but is proportional to the postfission variation.

In *Paramecium*, regulation of DNA content takes place during the period of DNA synthesis as is shown both by the decrease in the coefficient of variation of macronuclear DNA content during the interfission interval and by the transfer of all of the macronuclear DNA to the daughter cells (14). Thus, the present situation differs substantially from cases in which elimination of part of the excess DNA content through the formation of chromatin extrusion bodies during fission is part of the system of regulation of DNA content (5, 12).

Regulation of macronuclear DNA content during the period of DNA replication can be brought



about through two different classes of mechanisms. In the first type of system, partial "rounds" of DNA synthesis can occur so that the amount of DNA synthesized within an interfission period by a single macronucleus would not necessarily bear any fixed relation to a complete round or doubling of macronuclear DNA content. DNA synthesis is presumably controlled at the level of a macronuclear subunit which replicates completely. The total number of subunits which replicate is, however, controlled so that the total amount of macronuclear DNA is regulated. DNA synthesis presumably continues until either the total macronuclear DNA content or the amount of newly synthesized DNA reaches a level determined by a factor other than the initial DNA content of the cell.

Regulative DNA synthesis of this sort has been observed in the heterotrichous ciliate, *Bursaria truncatella* (27). In this organism, the daughter cells normally differ significantly in DNA content. During the interfission period, compensatory DNA synthesis occurs so that both cells achieve the same mean pre-fission DNA content. This mechanism is not applicable to *Paramecium*, however, because the present observations show that only half of the variation introduced at a particular fission is removed during the subsequent cell cycle. This suggests that in *Paramecium* the amount of DNA synthesized during a cell cycle rather than the total DNA content of the macronucleus is controlled and allows us to propose a formal model for DNA content regulation in *Paramecium*. This model (model I) is based on the observation that the variance and standard deviation of pre- and postreplication DNA contents are the same. The mean deviations are consequently also the same both before and after replication. We assume, in addition, that the deviations of the individual cells from the population mean are also the same both before and after replication. Thus,

$$x_1 = \bar{x}_1 - d \quad (1)$$

and

$$x_2 = 2\bar{x}_1 - d \quad (2)$$

where  $x_1$  and  $x_2$  are the pre- and postreplication DNA contents of the cell,  $\bar{x}_1$  is the mean prereplication DNA content of the population, and  $d$  is the deviation of the individual cell DNA content from the population mean. By solving expression

1 for  $d$ , substituting into expression 2, and simplifying we get:

$$x_2 = x_1 + \bar{x}_1. \quad (3)$$

Expression 3 suggests that the cells would be expected to synthesize a constant amount of DNA during each cell cycle, regardless of whether the initial DNA content was higher or lower than the population mean. Thus, cells with deficient initial DNA content would more than double their macronuclear DNA content and cells with excess initial DNA would synthesize less than a doubling of DNA. The deviations of the individual cell values from the mean would also decrease by one half from one cell cycle to the next if no new variation were introduced.

Alternatively, in the second class of models the DNA content is regulated through controlled variation in the number of complete rounds of DNA synthesis that occur within a single cell cycle. The number of doublings in DNA content that occur is determined by the initial DNA content of the macronucleus. Cells with an initial DNA content lower than a threshold level synthesize two complete doublings of DNA, whereas cells with 2 larger initial DNA content synthesize only a single doubling of DNA, and cells with an initial DNA content higher than an upper threshold level synthesize no DNA during the interfission interval. This model (model II) is based on observations of *Tetrahymena* (5, 6, 9) and has been proposed by Doerder and DeBault (9) to account for regulation of DNA in *Tetrahymena themophila* (= *T. pyriformis*, syngen 1) which does not normally form chromatin extrusion bodies. Elements of this model have also been suggested as parts of the regulatory process in cases in which there is regular elimination of DNA from the macronucleus during fission (5, 6, 12).

Three types of evidence make model II inappropriate for *Paramecium*. First, model II cannot account for the low variability of wild-type *Paramecium* DNA contents. The prereplication coefficient of variation (10%) is about half of the minimum coefficient of variation obtainable with model II (Fig. 4). Virtually the entire population of prereplication DNA contents in *Paramecium* falls between 80 and 125% of the mean. Thus, no nuclei would be small enough to undergo an additional round of replication and no nuclei would be large enough to fail to replicate, for the threshold levels for model II occur at about 70 and 140% of the mean, respectively. Therefore,

all cells would double their DNA content, regulation would not occur, and the variance of the population would increase until cells were produced with DNA contents sufficiently large or small to engage the regulative mechanism. Model II also predicts a postreplication DNA content variance that is significantly greater than that observed in wild-type *Paramecium* cells (Table V). Second, the DNA content variance does not increase during replication in *Paramecium* as expected by model II. Model II predicts that the coefficient of variation of DNA content should not change during replication (Fig. 4). In *Paramecium* it is halved (Fig. 3). And third, the replication process in *Paramecium* is essentially additive rather than multiplicative as model II suggests (Fig. 6). This last observation provides the strongest support for model I and eliminates models that require precise doublings of DNA content. DNA synthesis in *Paramecium* is not an all-or-nothing phenomenon at the level of the entire macronucleus as it appears to be in *Tetrahymena* (6).

Two further aspects of DNA synthesis and its regulation in *Paramecium* are consistent with model I and suggest that the overall rate of DNA synthesis (doubling time) should be different in cells with large and small macronuclei. First, *Paramecium* exconjugants can synthesize macronuclear DNA at rates up to three times as great as that observed in vegetative cells during macronuclear anlage development (3) or during the early stages of macronuclear regeneration (4). In both cases, the initial DNA content of the cells was lower than normal, suggesting that in normal cells the observed rate of DNA synthesis is not limited by the inherent rate of DNA replication but by other factors. Second, increase in the number of macronuclei or macronuclear anlagen per cell does not bring about an increase in the total predivision macronuclear DNA content (4),<sup>1</sup> and the rate of DNA synthesis per unit-volume of nucleus is reduced when extra nuclei are present (4), suggesting that only a limited amount of macronuclear DNA can be synthesized during a particular cell cycle.

The amount of macronuclear DNA that is synthesized by the cell during one cell cycle may be

<sup>1</sup> Morton, G. T., and J. D. Berger. 1978. Comparison of singlet and doublet *Paramecium tetraurelia*: DNA content, protein content and the cell cycle. *J. Protozool.* In press.

determined primarily by the mass of the cell. This idea is supported both by the very strong correlation between the mean DNA content and the mean cell mass in different *Paramecium* cell lines (13), including doublet cell lines which have approximately twice the normal DNA content and dry mass (see fn. 1), and by the observation that experimental increase in the cytoplasmic mass of *Tetrahymena* (11, 25) or *Paramecium* (J. D. Berger, unpublished results) cells through temporary blockage of either DNA synthesis or cytokinesis leads to a substantial increase in the amount of macronuclear DNA synthesized within a single cell cycle. However, doubling the mean postfission DNA content of *Paramecium* produces no change in the subsequent total protein content of the cells (J. D. Berger, manuscript in preparation). Taken together, these observations show that the mass of the cell has a much stronger effect on the DNA content than the DNA content has on cell mass, which in turn suggests that cell mass strongly influences the amount of DNA that can be synthesized within a single cell cycle. Thus, the theoretical precision of model I might be improved by assuming that the cell synthesizes a quantity of DNA that is proportional to the initial mass of the cell.

We thank Doctors Doerder, Frankel, Kimball, Cleffmann and Pasternak for helpful comments concerning the manuscript, and Doctors Doerder and DeBault for the use of their unpublished data.

This work was supported in part by grant A 6300 of the National Research Council of Canada to J. D. Berger. H. J. S. was supported by a Rotary International Graduate Fellowship.

Received for publication 20 May 1977, and in revised form 8 September 1977.

## REFERENCES

1. ALLEN, S., and I. GIBSON. 1972. Genome amplification and gene expression in the ciliate macronucleus. *Biochem. Genet.* **6**:293-313.
2. BERGER, J. D. 1971. Kinetics of incorporation of DNA precursors from ingested bacteria into macronuclear DNA of *Paramecium aurelia*. *J. Protozool.* **18**:419-429.
3. BERGER, J. D. 1973. Nuclear differentiation and nucleic acid synthesis in well-fed exconjugants of *Paramecium aurelia*. *Chromosoma (Berl.)*. **42**:247-268.
4. BERGER, J. D. 1973. Selective inhibition of DNA synthesis in macronuclear fragments in *Paramecium*

- aurelia* exconjugants and its reversal during macronuclear regeneration. *Chromosoma (Berl.)*. **44**:33-48.
5. CLEFFMANN, G. 1968. Regulierung der DNA-Menge im Makronukleus von *Tetrahymena*. *Exp. Cell Res.* **50**:193-207.
  6. CLEFFMANN, G. 1975. Amount of DNA produced during extra S phases in *Tetrahymena*. *J. Cell Biol.* **66**:204-209.
  7. CUMMINGS, D. J. 1975. Studies on macronuclear DNA from *Paramecium aurelia*. *Chromosoma (Berl.)*. **53**:191-208.
  8. DOERDER, F. P. 1973. Regulatory serotype mutations in *Tetrahymena pyriformis*, syngen 1. *Genetics*. **74**:81-106.
  9. DOERDER, F. P., and L. E. DEBAULT. 1975. Cytofluorometric analysis of nuclear DNA during meiosis, fertilization, and macronuclear development in the ciliate *Tetrahymena pyriformis*, syngen 1. *J. Cell Sci.* **17**:471-493.
  10. FAND, S. B. 1970. Environmental conditions for optimal Feulgen hydrolysis. In *Introduction to Quantitative Cytochemistry II*. G. L. Wied, and G. H. Bahr, editors. Academic Press, Inc. New York. 211-220.
  11. FRANKEL, J., L. M. JENKINS, and L. E. DEBAULT. 1976. Causal relations among cell cycle processes in *Tetrahymena pyriformis*: an analysis employing temperature-sensitive mutants. *J. Cell Biol.* **71**:242-260.
  12. FRENKEL, M. A. 1975. Regulation of macronuclear DNA content in the Holotrichous ciliate *Tillina magna* (Trichostomatida, colpodiadae). *Protistologica*. **11**:41-48.
  13. KIMBALL, R. F. 1967. Persistent intraclonal variation in cell dry mass and DNA content in *Paramecium aurelia*. *Exp. Cell Res.* **48**:378-394.
  14. KIMBALL, R. F., and T. BARKA. 1959. Quantitative cytochemical studies on *Paramecium aurelia*. II. Feulgen microspectrophotometry of the macronucleus during exponential growth. *Exp. Cell Res.* **17**:173-182.
  15. McDONALD, B. 1958. Quantitative aspects of desoxyribose nucleic acid (DNA) metabolism in an amiconucleate strain of *Tetrahymena*. *Biol. Bull. (Woods Hole)*. **114**:71-94.
  16. MORTON, P. R. 1977. Gene mutations affecting nuclear behavior in *Paramecium tetraurelia*. M.Sc. Thesis, University of British Columbia, Vancouver. 196 pp.
  17. RUIZ, F., A. ADOUTTE, M. ROSSIGNOL, and J. BEISSON. 1976. Genetic analysis of morphogenetic processes in *Paramecium*. I. A mutation affecting trichocyst formation and nuclear division. *Genet. Res.* **27**:109-122.
  18. RUTHMANN, A. 1964. Autoradiographische und mikrophotometrische Untersuchungen zur DNS-Synthese im Makronukleus von *Bursaria truncatella*. *Arch. Protistenkd.* **107**:117-130.
  19. RUTHMANN, A. 1972. Division and formation of the macronuclei of *Keronopsis rubra*. *J. Protozool.* **19**:661-666.
  20. SOKAL, R. R., and F. J. ROHLF. 1969. *Biometry*. Freeman, Cooper & Co., San Francisco.
  21. SONNEBORN, T. M. 1940. The relation of macronuclear regeneration in *Paramecium aurelia* to macronuclear structure, amitosis and genetic determination. *Anat. Rec.* **778** (Suppl.):53-54.
  22. SONNEBORN, T. M. 1954. Gene controlled aberrant nuclear behaviour in *Paramecium aurelia*. *Microb. Genet. Bull.* **11**:24-25.
  23. SONNEBORN, T. M. 1970. Methods in *Paramecium* research. In *Methods in Cell Physiology*. D. M. Prescott, editor. Academic Press, Inc., New York. 241-339.
  24. SONNEBORN, T. M. 1975. The *Paramecium aurelia* complex of 14 sibling species. *Trans. Am. Microsc. Soc.* **94**:155-178.
  25. WORTHINGTON, D. H., M. SALAMONE, and D. S. NACHTWEY. 1976. Nucleocytoplasmic ratio requirements for the initiation of DNA replication and fission in *Tetrahymena*. *Cell Tissue Kinet.* **9**:119-130.
  26. YAO, M. C., and M. A. GOROVSKY. 1974. Comparison of the sequence of macro- and micronuclear DNA of *Tetrahymena pyriformis*. *Chromosoma (Berl.)*. **48**:1-18.
  27. ZECH, L. 1966. Dry weight and DNA content in sisters of *Bursaria truncatella* during the interdivision interval. *Exp. Cell Res.* **44**:599-605.