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DOWNWARD REGULATION OF CELL SIZE IN *PARAMECIUM TETRAURELIA*: EFFECTS OF INCREASED CELL SIZE, WITH OR WITHOUT INCREASED DNA CONTENT, ON THE CELL CYCLE

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SUMMARY

Two temperature-sensitive cell-cycle mutants were used to generate abnormally large cells (size estimated by protein content) with either normal or increased DNA contents. The first mutant, *cc1*, blocks DNA synthesis, but allows cell growth at the restrictive temperature. The cells do not progress through the cell cycle while at the restrictive temperature, but do recover and complete the cell cycle when returned to permissive conditions. The progeny have increased cell size and normal DNA content.

Downward regulation of cell size occurs during the ensuing cell cycle at permissive temperature. Two processes are involved. First, the G_1 period is reduced or eliminated. As initial cell size increases there is a progressive shortening of the cell cycle to 75 % of normal. This limit cell-cycle duration is reached when the initial mass of the cell is equal to or greater than that of normal cells at the time of DNA synthesis initiation (0·25 of a cell cycle). Cells with the limit cell cycle begin macronuclear DNA synthesis immediately after fission. The durations of the *S* period and fission are normal. Second, the rate of cell growth is unaffected by the increase in cell size, and results in the partitioning of excess cell mass between the daughter cells at the next fission.

The second mutant, *cc2*, blocks cell division, but allows DNA synthesis to occur at a reduced rate so that cells with up to about 140 % of the normal initial DNA content and twice the normal cell mass can be produced. The pattern of cell-cycle shortening is the same as in *cc1*. The rates of growth and both the rate and amount of DNA synthesis are proportional to the initial DNA content. This suggests that the rates of growth and DNA synthesis are limited by the transcriptional activity of the macronucleus in both *cc1* and *cc2* cells when they begin the cell cycle with experimentally increased cell mass. Increases in both cell size and initial DNA content are required to bring about increases in the rates of growth and DNA accumulation.

INTRODUCTION

In typical eukaryotic cells the mitotic nuclear division mechanism ensures that daughter cells receive chromosomal complements that are quantitatively and qualitatively identical. The mechanism of cytokinesis is not so precise. Sister cells typically differ somewhat in cell mass (e.g. see Killander & Zetterberg, 1965*a*). Rates of growth and generation times also vary slightly. Thus, variation in cell mass is introduced during each cell cycle and, if there were no regulatory mechanisms, the variance of cell size within a cell population would increase without limit. This obviously does

not occur; cell mass shows only a limited variation (Killander & Zetterberg, 1965*a*; Killander, 1965; Kimball, 1967; Kimball, Perdue, Chu & Ortiz, 1971; Yen *et al.* 1975). Consequently, regulation of cell mass must occur in all organisms.

This paper examines the kinetics of cell-size regulation in *Paramecium* and its relation to the cell cycle. *Paramecium tetraurelia* is a relatively large protist with 50–100 times the volume of a typical diploid eukaryotic cell. It has a large polygenomic macronucleus, which can vary substantially in DNA content without the occurrence of genic imbalance. Cell size can also vary considerably. Although both cell size and macronuclear DNA content vary, there is usually a high correlation between mean cell size and mean DNA content (Kimball, 1967). These observations suggest that *Paramecium* can regulate cell size and that cell-size regulation and the regulation of macronuclear DNA content are related processes.

We have studied two temperature-sensitive (ts) cell-cycle mutants. The ts mutation *cc1* blocks macronuclear DNA synthesis, and consequently cell division at the restrictive temperature. However, protein synthesis and accumulation continue, so that cells with excess protein content can be produced. A second ts mutation, *cc2*, also blocks cell division, but allows macronuclear DNA synthesis to continue at the restrictive temperature, although at a reduced rate. The cells show an increase in both protein and DNA content at the restrictive temperature, although DNA content increases at a lower rate than protein content under these conditions.

These mutations make it possible to generate cells of increased size experimentally, with or without a concommitant increase in macronuclear DNA content. We were then able to examine the kinetics of downward regulation of cell size, and the roles of macronuclear DNA content and cell size in determining the rates of growth and DNA synthesis, cell-cycle duration and the changes in DNA and protein contents over the course of a recovery cell cycle following the return of the experimental cells to permissive conditions.

MATERIALS AND METHODS

Stocks and culture

P. tetraurelia (Sonneborn, 1975), was grown in phosphate-buffered grass medium (Sonneborn, 1970) with *Enterobacter aerogenes* as the food organism. Two mutant stocks derived from the wild-type stock 51-S were used. Stock d4-1002 carries the recessive ts mutation *cc1*, which blocks macronuclear DNA synthesis and cell division (Peterson & Berger, 1976). Stock d4-1003 carries the mutation *cc2*, which blocks cell division but not macronuclear DNA synthesis (Peterson & Berger, 1976).

Experimental design

Two groups of approximately 40 hand-synchronized cells were selected prior to fission. One group, designated the control sample, was incubated at the permissive temperature (27 °C). This group was used to determine the length of the normal cell cycle, and the protein and DNA content of untreated cells. The other sample, the experimental group, was incubated at 27 °C for 0·5 h before the start of heat treatment, to ensure that all cells had completed division before the beginning of the heat shock. Heat treatments were carried out in a water-bath with an electronic temperature controller, which maintained water temperature to ±0·02 deg. C. At the end of the heat treatment the cells were returned to the permissive temperature and

allowed to reach division. After the first division, one of the daughter cells was fixed, and the other was allowed to progress through the next cycle. After the second, both daughter cells were fixed. The cell cycle that included the heat treatment was designated the first cell cycle and the recovery cell cycle was the second one.

Cytochemical procedures

Newly divided cells were placed, by micropipette, on albumin-coated microscope slides and most of the culture medium was removed. This ensured maximum flattening of the cells as they dried. After drying the cells were fixed in ethanol/acetic acid mixture (3:1) for 30 min. RNA was removed by acid hydrolysis (5 N-HCl, 45 min, room temperature). Cells were then stained with the fluorochromes acriflavine HCl and primulin by the two-colour method of Cornelisse & Ploem (1976). Acriflavine stains DNA and primulin stains protein.

Quantitative fluorescence microscopy

Determinations of DNA and protein content of single cells were made with a Zeiss microfluorimeter. The excitation source was a mercury arc (HB-50). For measurement of acriflavine fluorescence, a 490 nm low-pass excitation filter, a 500 nm dichroic reflector and a 520 nm barrier filter were used. For measurement of primulin fluorescence, a 395 nm low pass excitation filter, 400 nm dichroic reflector and a 430 nm barrier filter were used. In addition a 490 nm low pass filter was placed above the barrier filter to eliminate any acriflavine fluorescence. Each fluorescence measurement was the average of 32 successive digitations (8 bit) of the amplified photomultiplier output. The measuring sequence began at a fixed interval (0·1 s) after the cells cells were first exposed to the exciting radiation.

Labelling and autoradiography

Labelling of *Paramecium* cells with [^{3}H]thymidine-labelled bacteria and autoradiography were carried out as described earlier (Berger, 1971).

Statistical procedures

Statistical procedures were performed according to Sokal & Rohlf (1969). Compound errors were estimated according to Beers (1953). Means are shown with their 95% confidence intervals.

RESULTS

Analysis of cc1*: increase in cell size alone*

Growth and DNA synthesis during heat treatment. At 34·4 °C, *cc1/cc1* cells were unable to synthesize macronuclear DNA. However, the rate of growth was only slightly less than that of wild-type cells at 34·4 °C (Peterson & Berger, 1976). Total cell protein increased linearly during heat treatment while DNA content remained at the G_1 level. The rate of growth (increase in protein) was about 75% of that of cells at 27 °C, the permissive temperature.

Cell-cycle duration. Heat treatment caused an extension of the first cell cycle equal to the duration of the heat treatment (open circles, Fig. 1). This suggests that *cc1* cells did not progress through the cell cycle while at the restrictive temperature when heat treatment was started early in G_1. The time between the end of the heat treatment and the subsequent fission did not vary with the duration of heat treatment. The duration of the experimental cell cycle less the duration of the heat treatment was consistently 90–100% of the control cell-cycle length. It is not known whether the

same effect occurs when heat treatment is started at other points in the cell cycle. The effects of heat treatment on the second cell cycle were quite different (filled circles, Fig. 1). There was a linear decrease in the length of the second cell cycle after heat treatments up to about 30% of the control cell-cycle length. With longer heat treatments a consistent cell-cycle duration of 75% of the control value was obtained.

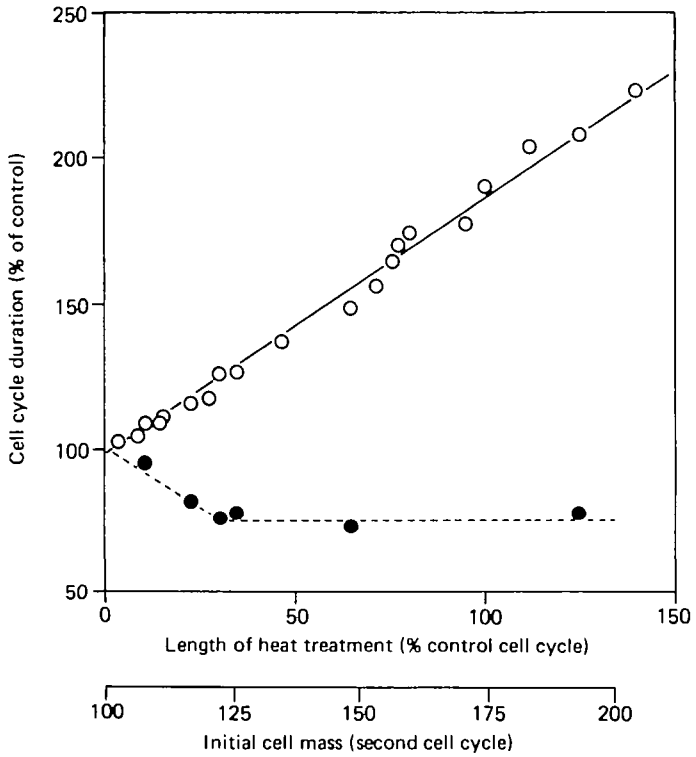

Fig. 1. Effect of heat treatment on the length of the 1st and 2nd cell cycles in *cc1/cc1* cells. (○) 1st cell cycle; (●) 2nd cell cycle. Cell mass is expressed as a percentage of the initial mass of control cells.

DNA content and DNA synthesis. Heat treatment of *cc1* cells during the first cell cycle had no effect on macronuclear DNA content (open circles, Fig. 2). The post-fission DNA content never differed significantly from that of the control sample. Thus, *cc1* cells synthesized the normal amount of DNA after they were returned to the permissive temperature, even though their cell sizes were up to twice the normal size. The DNA content of *cc1* cells was still at the normal level following the second cell cycle after heat treatment (filled circles, Fig. 2). Although the second cell cycle was shortened by 25%, the normal amount of DNA was made, despite increased cell size.

Cell size and growth rate. Since growth continued during heat treatment, *cc1* cells were: (1) larger than normal at the end of the first cell cycle; and (2) the increase in cell mass was directly proportional to the length of the heat treatment (open circles, Fig. 3). By the end of the second cell cycle, *cc1* cells were smaller than at the end of the first cell cycle (filled circles, Fig. 3). The greater the initial cell mass, the greater

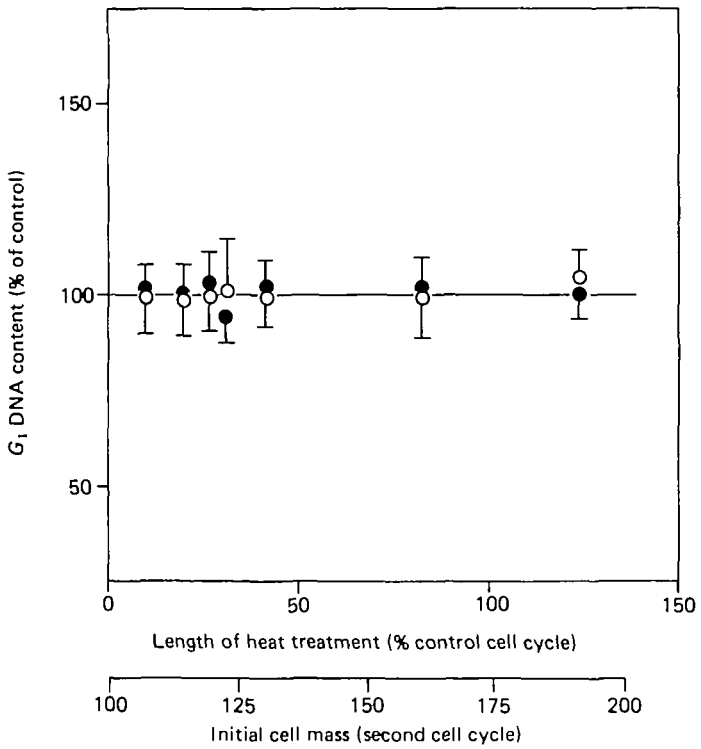

Fig. 2. G_1 macronuclear DNA content in heat-treated *cc1/cc1* cells. (○) Start of 2nd cell cycle; (●) start of 3rd cell cycle. Cell mass is expressed as a percentage of the initial mass of control cells.

the degree of reduction during the second cell cycle. The amount of protein synthesized during the second cell cycle closely parallels the pattern of decrease in cell-cycle duration as initial cell mass increased (compare filled circles, Fig. 4 and Fig. 1). Thus, although the amount of protein synthesized in cells with different initial masses varied because of differences in the length of the cell cycle, the rate of protein accumulation was constant and independent of initial cell mass.

Analysis of cc2*: increase in both cell size and DNA content*

At 34·25 °C, *cc2/cc2* cells did not undergo cell division. However, unlike *cc1*, the *cc2* cells synthesized macronuclear DNA at the restrictive temperature. Cell mass increased linearly throughout heat treatment at 77 % of the normal rate. Macronuclear DNA content also increased linearly, but only when heat treatment extended past 0·25 of the cell cycle. This point in the cell cycle coincides with initiation of DNA synthesis in normal cells (Berger, 1971).

Cell-cycle lengths. The first cell cycle was extended by heat treatment (open circles, Fig. 5). However, unlike *cc1* cells described above, the increase in cell-cycle duration was less than the length of the heat treatment. The time between the end of heat treatment and division decreased as the duration of heat treatment increased (Fig. 6).

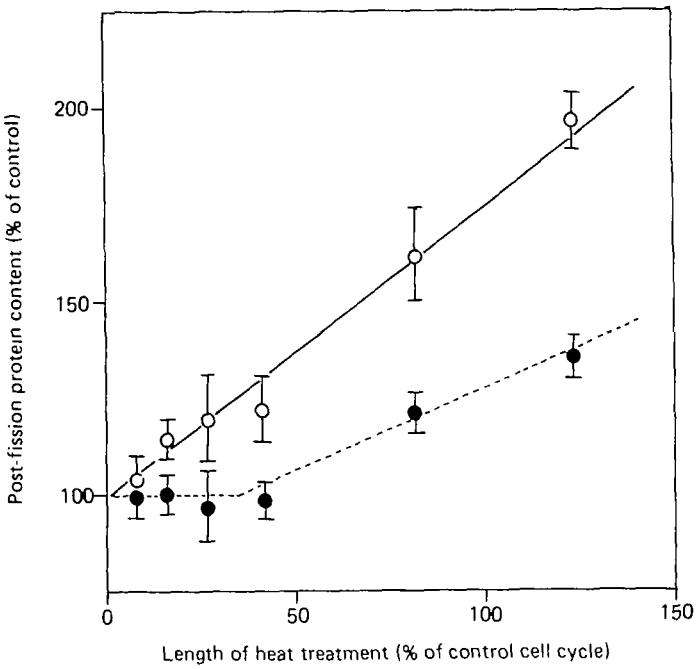

Fig. 3. Post-fission protein content in heat-treated *cc1/cc1* cells. (○) Start of 2nd cell cycle; (●) start of 3rd cell cycle.

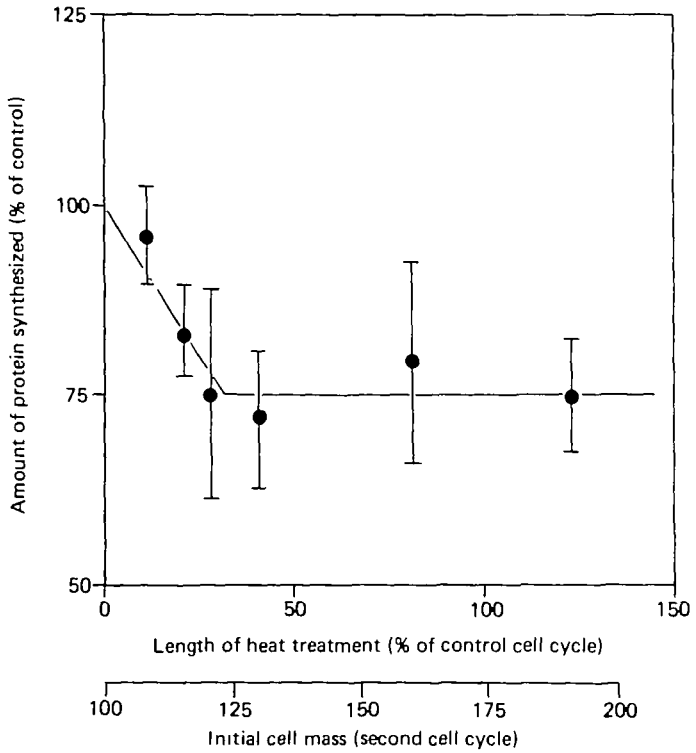

Fig. 4. Protein accumulation during the 2nd cell cycle in heat-treated *cc1/cc1* cells. Cell mass is expressed as a percentage of the initial mass of control cells.

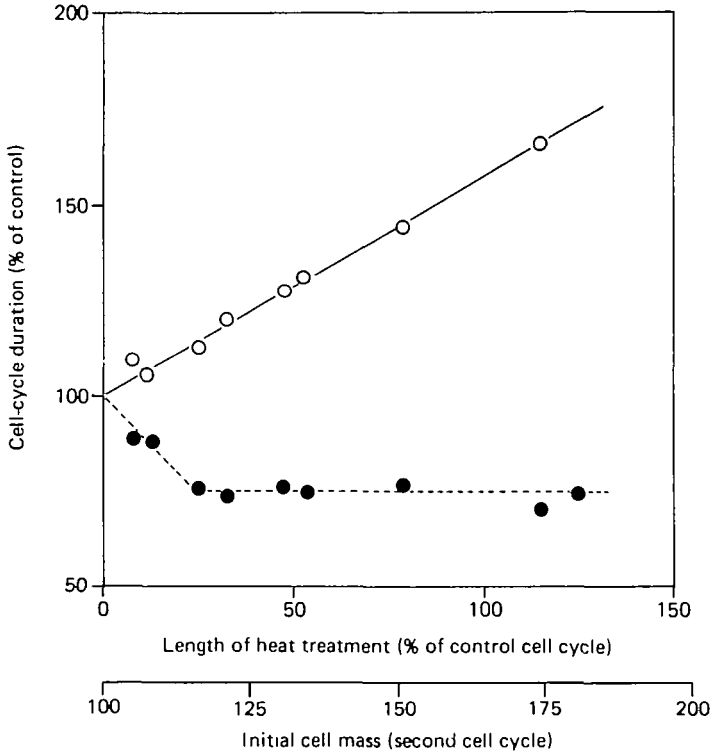

Fig. 5. Effect of heat treatment on the length of the 1st and 2nd cell cycles in *cc2/cc2* cells. (○) 1st cell cycle; (●) 2nd cell cycle. Cell mass is expressed as a percentage of the initial mass of control cells.

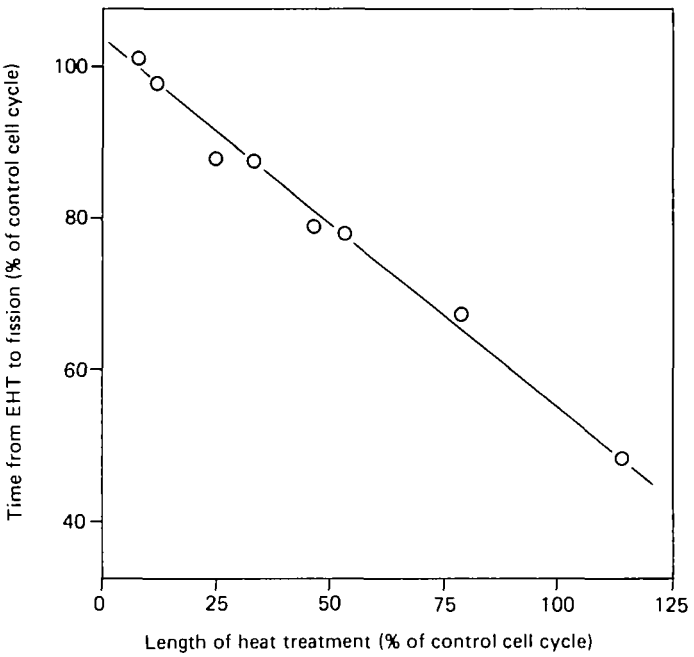

Fig. 6. Time required for *cc2/cc2* cells to complete the cell cycle after the end of heat treatment (EHT).

This suggests that *cc2* cells progress through the cell cycle while at the restrictive temperature, but at only about 40% of the normal rate. The duration of heat treatment also significantly affected the duration of the second cell cycle. The pattern was very similar to that of *cc1* cells. There was a linear decrease in the duration of the second cell cycle with heat treatments up to 30% of normal cell-cycle length. Longer heat treatments produced a second cell cycle 75% of the normal length.

DNA synthesis. Heat treatments restricted to the G_1 portion of the first cell cycle caused no significant change in DNA content. However, when heat treatment extended into the period of macronuclear DNA synthesis, DNA content increased

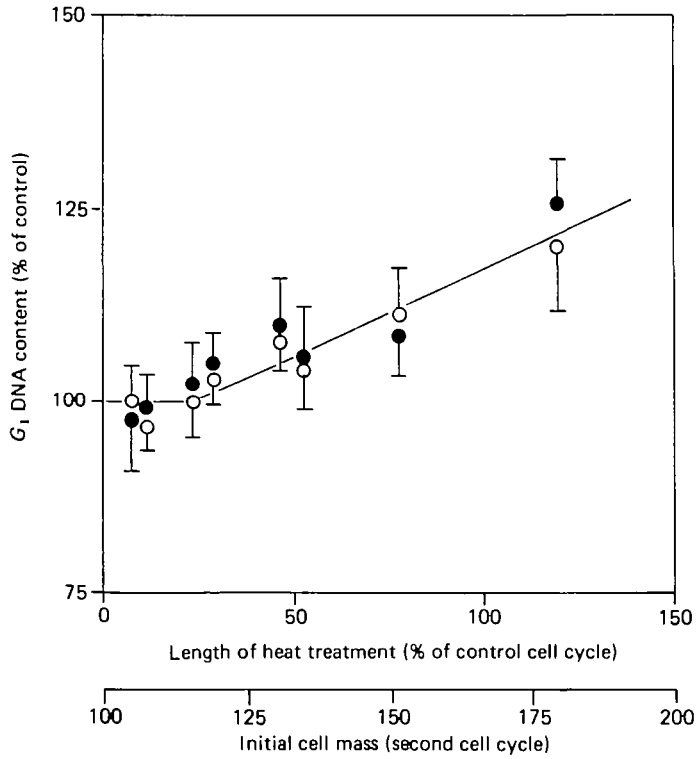

Fig. 7. G_1 macronuclear DNA content in heat-treated *cc2/cc2* cells. (○) Start of 2nd cell cycle; (●) start of 3rd cell cycle. Cell mass is expressed as a percentage of the initial mass of control cells.

(Fig. 7). The rate of increase in DNA content was about 20% of normal. Thus, although DNA synthesis occurred in *cc2* cells at the restrictive temperature, the rate was reduced. During the second cell cycle there was a strong correlation between the initial DNA content and the rate of macronuclear DNA synthesis (r = 0·97).

Cell mass. Like *cc1*, *cc2* cells had an extended cell cycle at the restrictive temperature. The cells grew normally and were thus larger than normal at the end of the first cell cycle (open circles, Fig. 8). The increase in cell size was directly proportional to the duration of heat treatment.

Reduction in cell size occurred during the second cell cycle (filled circles, Fig. 8).

Cells heat-treated entirely within the G_1 period of the first cell cycle regained normal size, while those given heat treatments longer than 0·3 of a cell cycle retained greater than normal cell mass. The absolute amount of cell-mass reduction during the second cell cycle increased with increasing initial cell mass.

Unlike *cc1* cells, in which the rate of growth during the second cell cycle was constant, the rate of growth in *cc2* cells showed a strong positive correlation between G_1 DNA content and growth rate (r = 0·92). This observation and the fact that the macronuclear DNA content was always less than the initial protein content suggests that macronuclear gene dosage is the growth-limiting variable where cell mass is relatively greater than macronuclear DNA content.

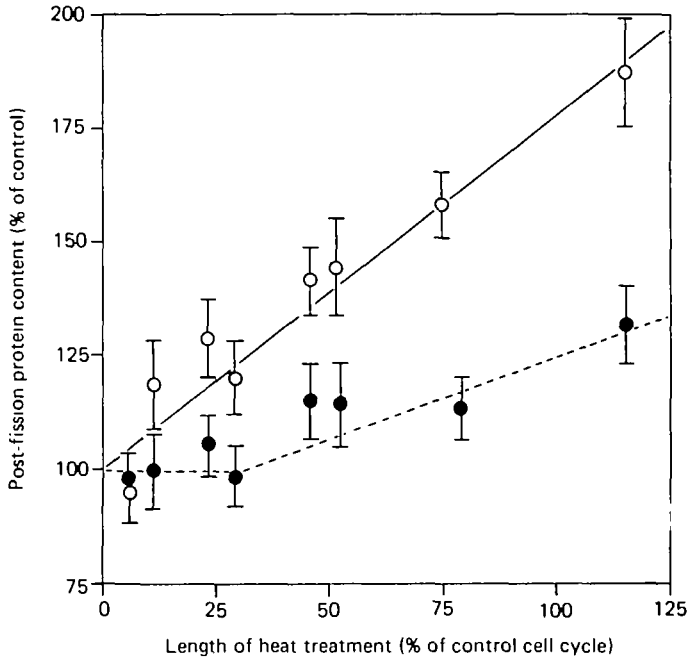

Fig. 8. Post-fission protein content in heat-treated *cc2/cc2* cells. (○) Start of 2nd cell cycle; (●) start of 3rd cell cycle.

Initiation of DNA synthesis in experimentally enlarged cells

When *Paramecium* cells begin the cell cycle with increased cell mass, the cell cycle is shortened. If the initial cell mass is 120% of normal, or greater, the duration of the cell cycle is reduced by an amount equal to the normal duration of the G_1 period. This suggests that initiation of DNA synthesis immediately follows fission in cells with minimum cell-cycle duration. This idea was tested by examining initiation of DNA synthesis in both normal (not heat-treated) and experimentally enlarged *cc1* cells. Enlarged dividing cells were obtained by exposing synchronous *cc1* cells to a 3 h heat treatment beginning 0·5 h after fission. The cells were then allowed to complete the cell cycle at the permissive temperature. These experimental cells produced daughter cells with a mean protein content of 133% of the control value. Synchronous samples

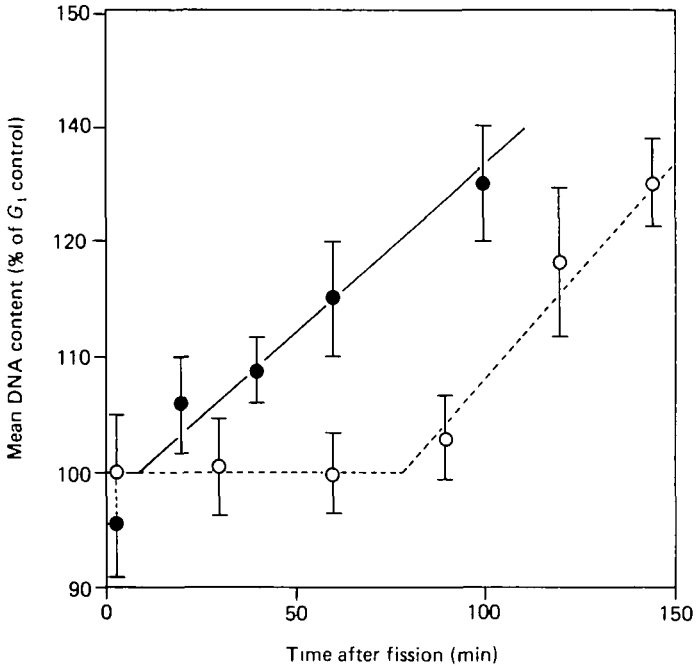

Fig. 9. Macronuclear DNA content after fission in normal size (○) and experimentally enlarged (●) *ccl/ccl* cells.

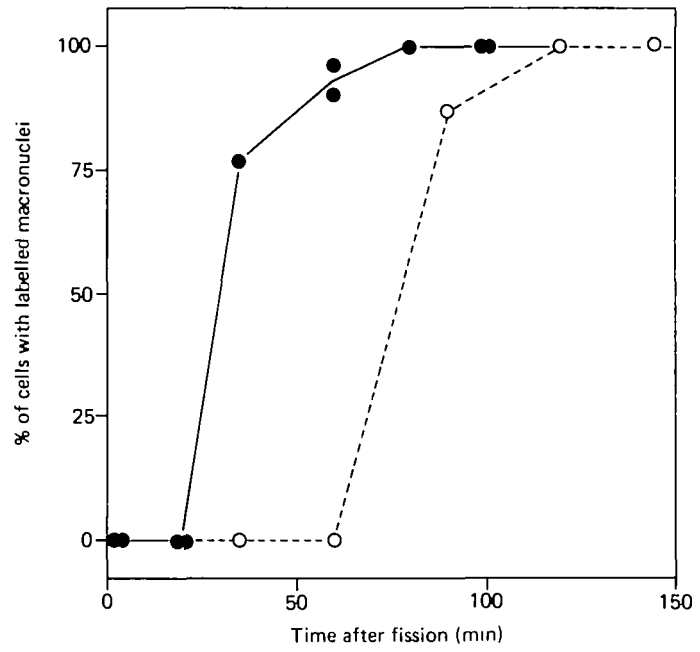

Fig. 10. Percentage of cells showing significant incorporation of [^{3}H]thymidine after fission in normal (○) and experimentally enlarged cells (●).

of enlarged daughter cells were selected and tritiated thymidine was added immediately after fission. Cells were withdrawn periodically and fixed. The mean DNA content and the fraction of macronuclei showing incorporation of tritiated thymidine were then determined. A parallel experiment was also carried out with cells that were not heat-treated.

In experimental cells, increase in the mean macronuclear DNA content began immediately after fission (Fig. 9) and incorporation of tritiated thymidine began at about 20 min after transfer of the cells to labelled medium (Fig. 10). This lag is consistent with the observations that food vacuoles are not formed until about 15 min after fission (W. R. Jones, 1976), and that approximately 5 min is required for food-vacuole formation and incorporation of labelled material into macronuclear DNA (Berger, 1971). In contrast, increase in macronuclear DNA content and incorporation of tritiated thymidine in control cells with normal mass did not begin until about 78 min after fission, which represents 0·25 of a cell cycle.

DISCUSSION

Regulation of cell mass in *Paramecium* involves the concerted action of two processes, which control: (1) the rate of cell growth and DNA accumulation; and (2) the duration of the cell cycle. Both processes are linked to different aspects of the macronuclear DNA replication cycle. The first appears to involve gene-dosage limitation of the rates of growth and DNA accumulation; the second cell-mass-dependent control of DNA synthesis initiation.

Control of growth and DNA accumulation

This study shows that increased cell mass does not lead to increase in the rates of growth or DNA accumulation when macronuclear DNA content remains at the normal level. Experimental increase in macronuclear DNA content also produces little or no subsequent change in the rates of growth or DNA accumulation when cell mass remains unchanged (Berger, 1978, 1982*b*). On the other hand, coordinated increases in both variables result in proportional increases in the rate of growth and in the rate of DNA accumulation. This suggests that the rates of growth and DNA accumulation are limited by either cell mass or DNA content. When either DNA content or cell mass is increased, the other variable becomes rate-limiting. This is also true when both DNA content and cell mass are increased; the rates of growth and DNA accumulation increase to a value proportional to the lower of the two variables. The rates of both cell growth and DNA accumulation are also greatly decreased in cells with experimentally reduced DNA content (Berger, 1979). The regulative interaction of DNA content and cell mass appears to be responsible for both the strong correlation observed between mean DNA content and mean cell mass in *Paramecium* (Kimball, 1967; Morton & Berger, 1978) and the regulation of cell mass and DNA content.

Some elements of the physiological basis of this regulatory system have been elucidated by recent observations on the rates of protein synthesis in *Paramecium*. At low DNA contents, the rate of protein synthesis is directly proportional to DNA

content (Berger, 1982*a*). This proportional response persists as DNA content (gene dosage) increases past the normal level to a critical gene dosage (average 8% higher than mean DNA content) at which a maximum rate of protein synthesis is reached. Further increase in gene dosage has no significant effect on the rate of protein synthesis. Further, the critical gene dosage is reached at a characteristic 'gene concentration' (gene dosage/cell mass; Fraser & Nurse, 1979), which remains constant throughout the cell cycle. As the cell grows, increasing gene dosage is required to reach the critical gene concentration that produces the maximum rate of protein synthesis.

Our working hypothesis is that the rate of protein synthesis is limited by availability of DNA template when gene dosage is below the critical level, as it is in cells with normal or reduced DNA content. Above the critical gene dosage the rate of protein synthesis is limited, possibly by saturation of the protein-synthesizing system, or by a concentration-dependent feedback system that regulates the rate of transcription so that a constant concentration of gene transcripts is maintained in the cytoplasm. The available data are insufficient to eliminate either hypothesis. A concentration-dependent feedback system has been proposed as the basis of cell-size regulation in the fission yeast, *Schizosaccharomyces* (Fraser & Nurse, 1978, 1979; Barnes, Nurse & Fraser, 1979). We favour the simpler saturation hypothesis in the absence of evidence to the contrary.

The present observations are consistent with either of these alternative models if it is assumed that the rate of protein synthesis limits both the rate of DNA accumulation and the rate of cell growth. This notion remains unproven. While it is consistent with all of the observations, other alternatives cannot be excluded.

Control of cell-cycle length

The second process contributing to the regulation of cell mass in *Paramecium* involves shortening of the cell cycle in cells with increased cell mass. As initial cell mass increases there is a progressive shortening of the cell cycle to 75% of its normal duration. Further increase in initial cell mass has no effect on cell-cycle length. Since DNA synthesis initiation begins at 0·25 in the cell cycle (Berger, 1971) the minimum cell-cycle length suggests that the G_1 period is deleted as initial cell mass increases. If DNA synthesis initiation is cell-mass dependent, the initial cell mass required for minimum cell-cycle duration (118–120% of normal level) should be the same as that normally present at initiation of DNA synthesis. Cell mass at the normal time of DNA synthesis initiation is normally about 120% of the initial value (Kimball, Caspersson, Svensson & Carlson, 1959; Berger, 1982*a*).

These observations suggest that in *Paramecium* DNA synthesis begins when cell mass reaches 120% of the normal initial level. Consequently, there is virtually no G_1 period if initial cell mass equals or exceeds this threshold level, as shown by the present labelling experiments. Reduction or elimination of the G_1 period following the experimental production of abnormally large cells through blockage of DNA synthesis has also been observed in *Tetrahymena* (Worthington, Salamone & Nachtwey, 1976) and in some studies on mammalian cells (Gerner, Meyn & Humphrey,

1976; Cress & Gerner, 1977). It is not clear whether initiation of DNA synthesis in eukaryotes is strictly determined by cell mass, or is a consequence of growth (Liskay, Kornfeld, Fullerton & Evans, 1980).

A larger body of experimental evidence suggests that many cell types have relatively rigid cell-mass requirements for initiation of DNA synthesis and that the G_1 period in small cells is more extensive than in larger cells (e.g. see Johnston, Pringle & Hartwell, 1977; Nurse & Thuriaux, 1977; Killander & Zetterberg, 1965*a*, *b*; Kimball *et al.* 1971; Yen *et al.* 1975). Because the present experiments have examined downward regulation of cell mass only it is not known whether the cell-mass requirement for initiation of DNA synthesis in *Paramecium* is absolute; that is, whether cells that are smaller than normal are unable to enter *S* phase until they reach the normal mass required for initiation. Two lines of evidence suggest that cells that are smaller than normal are able to initiate DNA synthesis. First, cells with normal initial mass, but severely decreased gene dosage initiate DNA synthesis at the normal time (Berger, 1982*b*). The rates of protein synthesis and cell growth in these cells are also highly reduced, so that the cells are smaller than normal at the time of DNA synthesis initiation (Berger, 1982*a*). Second, the cell cycle is maintained in well-fed cells with reduced cell mass. The temperature-sensitive mutation *sm2* (Jones & Berger, 1982) interferes with cell-surface proliferation. At the restrictive temperature cells continue to traverse the cell cycle, but become progressively smaller with each successive fission.

The two regulative processes revealed by this study – limitation of growth rate and shortening of the cell cycle in enlarged cells – act to attenuate quickly experimental increases in cell mass. The limitation of the rate of growth to normal levels when cell mass is increased results in the loss of 50% of the excess cell mass per cell cycle. The rate of growth is insufficient to double the increased cell mass and the excess material is partitioned into the daughter cells. The shortened cell cycle is responsible for a further reduction of up to 25% in cell mass. A computer-simulation model of the combined regulatory processes produces a good fit to the observed consequences of experimental increases in cell mass (Berger, 1982*a*) and suggests that increases in cell mass of up to 20% are attenuated within one cell cycle. Since the coefficient of variation of cell mass is normally $\sim 20\%$ (Kimball *et al.* 1959; Berger, 1978), most or all normal variation in cell mass is removed within one cell generation of its occurrence.

This study suggests that cell-size-dependent initiation of DNA synthesis is an important component of cell-cycle regulation in *Paramecium* and may be the major set point for regulation of cell size. Without the reduction of cell-cycle duration in cells with increased cell mass and increased DNA content (such as heat-treated *cc2* cells) the increased cell mass and DNA content would be maintained, for the rates of growth and DNA accumulation are also proportionately increased. It is apparent that the effect of increased cell mass on the timing of DNA synthesis initiation is not merely a function of absolute cell mass, for doublet cells have twice the normal cell mass and still begin DNA synthesis at 0·25 of the cell cycle (Morton & Berger, 1978). Incomplete doublets are smaller, but still maintain cell and nuclear sizes that are greater than those in normal singlet cells (Sonneborn, 1963). This suggests that the size of the cell

cortex plays a role in determination of the set point for regulation of cell mass and DNA content. The occurrence of relatively stable cortical variants (corticotypes) with altered numbers of kinetics and proportionate increases or decreases in cell surface (Nanney, 1966; Frankel, 1980) may be the basis of the occasional long persistence of cell lines with varying cell mass and DNA contents (Kimball, 1967).

The shortening of the cell cycle observed in enlarged cells is also not simply a consequence of reduction of gene dosage (gene dosage/cell mass; Fraser & Nurse, 1979). In cells with normal cell mass, variation in gene dosage in the post-fission period had no effect on the timing of DNA synthesis initiation with normal cell mass. This suggests that the conditions regulating initiation of DNA synthesis are established prior to the preceding fission in *Paramecium*, unlike the situation in *Tetrahymena* (Seyfert, 1977; Cleffmann, Reuther & Seyfert, 1979).

A control mechanism is also required for cell-cycle termination. In *Paramecium* this system is not time-dependent, for cells with highly decreased gene dosage require three to five times the normal cell-cycle duration to reach fission (Berger, 1979), even though the timing of DNA synthesis initiation is normal (Berger, 1982*b*). The observations that these cells synthesize the normal amount of DNA (Berger, 1979) and reach at least normal cell mass at division suggest that cell division depends on growth and DNA synthesis in *Paramecium* and may also be cell-mass-dependent as observed in fission yeast (Fantes & Nurse, 1977).

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