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THE CELL CYCLE OF SYMBIOTIC *CHLORELLA*

III. NUMBERS OF ALGAE IN GREEN HYDRA DIGESTIVE CELLS ARE REGULATED AT DIGESTIVE CELL DIVISION

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SUMMARY

Regression analysis of the relationship between the size of interphase and mitotic digestive cells of green hydra, and the numbers and total volume of the symbiotic *Chlorella* algae they contain showed a partial correlation only, suggesting that numbers of algae per cell are not regulated by limiting them to a specific proportion of the host cell, and that the variation observed in numbers of algae per cell is not due to variation in host cell size. After hydra were fed, which stimulates algae and digestive cells to divide at the same time, numbers of algae per cell were higher in prophase than in interphase cells, and numbers increased as mitosis proceeded. In excised regenerating peduncles algae divide before digestive cells, and at the onset of digestive cell division mitotic cells were found to contain almost twice the number of algae as before excision. Thus, almost all of the algal cell division necessary to maintain a constant population size was associated with digestive cell division. Analysis of variance in numbers of algae in telophase mother and daughter cells suggested that the proportion of algae dividing as a result of host cell mitosis was greater in digestive cells with few algae than in those with many algae. The fact that the mechanism controlling the proportion of algae dividing in host cells is expressed at host cell division and is manifested in the daughter cells may contribute to wide variation in numbers of algae per cell.

INTRODUCTION

When green hydra are maintained in constant environmental conditions, the size of the symbiotic algal population per animal remains at a constant level (Pardy, 1974; McAuley, 1981*a*,*b*; Douglas & Smith, 1984); if environmental conditions change, the size of the algal population also changes (for review, see McAuley, 1985*a*). The algae are contained within individual vacuoles at the bases of endodermal digestive cells. On the basis of average number of algae per cell, Pardy (1974) divided the body column into three parts; cells in the head and tentacle region and in the peduncle region both containing fewer algae than those in the gastric and budding region. Differences may be due to differences in metabolic and mitotic activity (Pardy, 1974) or in cell volume (Douglas & Smith, 1984). However, while in any of the three regions the *average* number of algae per cell is constant in constant conditions, there is a very wide variation between digestive cells of the same population (Pardy, 1974; McAuley, 1981*a*). It is not known whether this variation corresponds to host cell differences (i.e. cell size) or whether there are other factors leading to unequal assortment of algal cells between digestive cells, such as an inherent variation

Key words: green hydra, *Chlorella*, symbiosis, mitosis.

produced by the control mechanism that regulates algal cell division (McAuley, 1981*a*, 1985*b*). The aim of this paper is to discriminate between these possibilities.

Muscatine & Pool (1979) proposed that symbiotic algae are restricted to a certain proportion ('lebensraum') of the host cell. This has a parallel in higher plant cells, in which there is a positive correlation between cell size and number of chloroplasts (Boasson *et al.* 1972; Kameya, 1972; Paolillo & Kass, 1977; Tsuji *et al.* 1979; Scott & Possingham, 1980; Whatley, 1980; Wild & Wolf, 1980; Asahi & Toyama, 1982; Olszewska *et al.* 1983; Ellis & Leech, 1985). Douglas & Smith (1984) analysed the correlation between digestive cell volume and the volume of algae contained in digestive cells in European green hydra grown in constant light, 12 h light/12 h dark or constant darkness. Although in all cases a significant correlation was obtained, only 50 % or less of the variation in total algal volume per cell could be attributed to digestive cell size.

Previous work has shown that algal cell division is entrained to that of the digestive cells in which they reside (McAuley, 1981*a*, 1982, 1985*b*). However, to maintain a constant population of algae only a proportion needs to divide at host cell mitosis, since digestive cells produce two daughter cells but an alga produces an average of just over four at cell division. It is not known what determines the proportion of algae that divide at host cell mitosis, or whether there is also algal division associated with host cell growth, as would be expected if algal volume is regulated to host cell size. These two factors have obvious implications in the regulation of the algal population size in digestive cells.

In this paper, observations on the size of algal populations in interphase and mitotic cells suggest that there is no direct relationship between host cell size and either the number or the total volume of algae the cells contain. Almost all algal division is associated with host cell mitosis, and a model is proposed that describes how the proportion of algae that divide at host cell mitosis is determined.

MATERIALS AND METHODS

Experimental organisms

Stock cultures of the 'European' strain of *Hydra viridissima* were maintained as previously described (McAuley, 1985*b*). All hydra used in experiments had been fed 24 h previously and each bore a single bud.

Measurement of cell size

Pieces of hydra were placed on a glass slide and incubated for 10 min in a drop of macerating fluid (David, 1973) containing 5 $\mu g\,ml^{-1}$ of the DNA-specific fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) before being teased apart into a suspension of individual cells with a fine steel needle. Macerates were covered with a coverslip and kept at 4°C for 24 h to enable the cells to flatten out; they were examined by a Leitz Dialux EB20 microscope using ×500 fluorescence optics to identify mitotic and non-mitotic cells.

Size of digestive cells was estimated by determining the area of flattened cells from traced outlines and multiplying by an estimated depth to give cell volume (Douglas & Smith, 1984). Outlines of flattened digestive cells in macerates were traced onto paper at a final magnification of ×625, using Nomarski optics and a drawing attachment, then cut out and weighed on a Sartorius

1601 A MP8-1 electronic balance with an accuracy of 0·1 mg. Magnification at the surface of the paper was determined accurately using a graticule (Graticules Ltd, London), and weights of paper were converted to digestive cell surface area from known weight per unit area, determined by weighing 10 units of 10 cm^2 in area. Estimates of cell depth were obtained from the distance between focal points on the upper and lower surfaces of digestive cells; all were between 9 and 12 μm and an average of 10 μm was used (Douglas & Smith, 1984). Depth was assumed to be equal over the whole area of flattened cells chosen for measurements.

Macerating fluid rapidly fixes hydra cells and does not affect cell architecture, but may cause some swelling due to the acetic acid it contains; macerated cells are about 30 % larger than cells in formaldehyde-fixed sections of hydra (David, 1973). However, Bosch & David (1984) suggested that this did not affect the validity of comparing sizes of cells in macerates. They found that cells in S-phase, at the beginning of the cell cycle, were smaller than those in G_2, while cells in metaphase were roughly twice the size of telophase daughter cells. In this paper, the sizes of digestive cells at the beginning and end of mitosis were very similar, 11 410 ± 420 μm^3 and 11 540 ± 420 μm^3, respectively, despite differences in cell architecture: prophase cells are roughly cylindrical while telophase cells are dumbell-shaped. The size of digestive cells at interphase was within the range previously measured by Douglas & Smith (1984) in this strain of green hydra.

Diameters of algae within digestive cells were indicated separately and used to calculate algal cell volume by assuming the algae to be perfect spheres. Measurements were made in 50 interphase and telophase cells, and 36 prophase cells, in eight replicate macerations of five gastric regions of hydra fed 24 h previously.

RESULTS

Relationship between host cell size and size of algal population

The mean cell volumes of mitotic and interphase cells from gastric regions of fed hydra were similar (Table 1), and interphase and telophase cells showed the same distribution of sizes (Fig. 1). Indeed, the sizes of a large proportion of daughter cells produced at telophase overlapped the lower range of telophase mother cell size, suggesting that cell volume does not play a major role in regulation of digestive cell division.

The total volume of the algal population, and the proportion of digestive cell volume occupied by the algae in mitotic cells, was about twice that of interphase cells (Table 1). Increased numbers of algae in mitotic digestive cells confirm previous

Table 1. *Parameters of algal symbiosis in dividing and non-dividing digestive cells 24 h after feeding*

Stage	Total digestive cell volume (μm^3)	Total volume of algae (μm^3)	Algae per digestive cell	Mean algal cell volume (μm^3)	% Digestive cell occupied by algae*
Interphase (50)	11 340 ± 570	1280 ± 42	13·78 ± 0·88	93·45 ± 4·73	12·7
Prophase (36)	11 410 ± 420	2276 ± 126	18·72 ± 0·83	122·55 ± 4·66	24·8
Telophase (50)	11 540 ± 420	2290 ± 130	20·22 ± 0·93	105·33 ± 5·06	24·8

Values are means ± S.E.; number of observations is given in parenthesis.

* Given by $\frac{V_a}{V_t - V_a} \times 100$, where V_a is total volume of algae and V_t total volume of digestive cell.

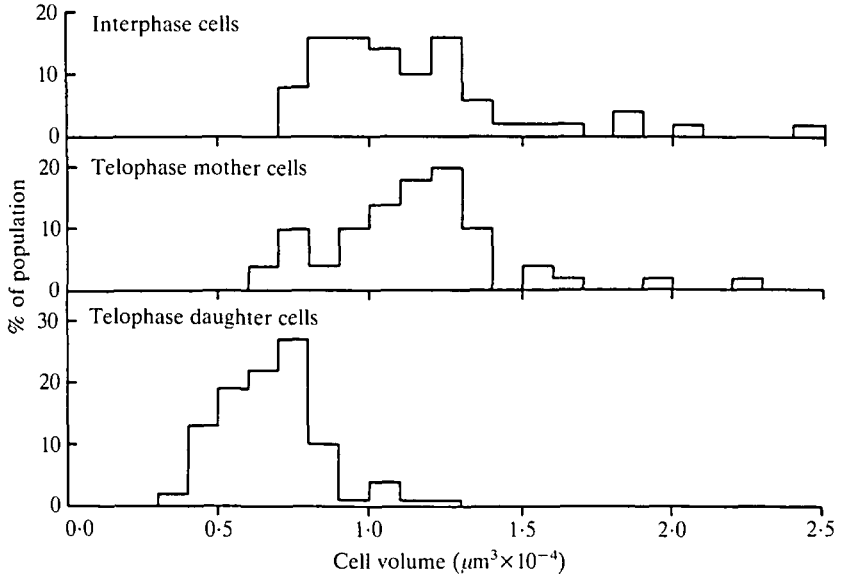

Fig. 1. Distribution of cell volume of interphase, telophase mother and telophase daughter digestive cells.

suggestions that algal cell division begins when the host cell is stimulated to divide (McAuley, 1981*a*, 1982). Mean algal cell volume in prophase cells was higher than in interphase cells, perhaps because digestive cells at the start of mitosis contained algae that had been growing in size after a previous round of cell division. Algal cell size was somewhat reduced in telophase digestive cells, presumably as a result of cell division producing smaller daughter cells.

The total volume of algae per cell was only partly correlated with number of algae (Table 2). While analysis of variance of the regression of total volume on numbers of algae showed that there was a significant relationship ($P < 0{\cdot}001$), the proportion of variance of total algal volume that could be attributed to linear regression on numbers of algae was 50 % or less (given by r^2). This may have been due to the relatively small numbers of algae per cell and the high variation in algal cell size (Pardy, 1981; McAuley, 1985*b*). Accordingly, the total algal volume per cell was calculated from individual measurements of algae in each cell rather than by simply multiplying numbers of algae per cell by mean algal cell volume (Douglas & Smith, 1984).

Parameters of linear regression of individual measurements of numbers of algae and total algal volume per cell on digestive cell size are given in Table 3. Analysis of

Table 2. *Linear regression of number of algae on total algal volume per cell*

	Regression equation	F_1, $(n-1)$	r^2
Interphase	$y = 344{\cdot}57 + 67{\cdot}16x$	51·96	0·52
Prophase	$y = 303{\cdot}56 + 105{\cdot}15x$	31·81	0·48
Telophase	$y = 331{\cdot}52 + 86{\cdot}53x$	31·55	0·40

Table 3. *Linear regression of digestive cell volume on numbers of algae and total algal volume per cell*

	Regression equation	$F_{1,}$ $(n-1)$	r^2
Interphase			
Cell vol.: algal no.	$y = 0{\cdot}00088x + 3{\cdot}837$	23·83	0·332
Cell vol.: algal vol.	$y = 0{\cdot}098x + 166{\cdot}7$	45·18	0·485
Prophase			
Cell vol.: algal no.	$y = 0{\cdot}00135x + 3{\cdot}349$	29·32	0·463
Cell vol.: algal vol.	$y = 0{\cdot}185x + 161{\cdot}4$	20·99	0·382
Telophase			
Cell vol.: algal no.	$y = 0{\cdot}00128x + 5{\cdot}468$	24·49	0·338
Cell vol.: algal vol.	$y = 0{\cdot}125x + 639{\cdot}6$	9·86	0·170

variance showed that in all cases regression was significant ($P < 0{\cdot}001$), but r^2 showed that regression on digestive cell size accounted for less than half the variation in numbers of algae and total algal volume in interphase, prophase and telophase cells. Total algal volume showed no better correlation with host cell volume than numbers of algae.

Size of algal population during digestive cell mitosis

Mitosis of digestive cells was stimulated either by host feeding or by excising peduncles and allowing them to regenerate, and numbers of algae were counted in prophase, metaphase, anaphase and telophase stages. After host feeding, digestive cell and algal mitotic indices increase in parallel (McAuley, 1982). Counts of numbers of algae at different stages of mitosis (Table 4) suggested that division of the host cell directly stimulated division of the algae, since numbers increased until telophase. The variance ratio, derived from analysis of variance of the four populations of algae (Table 5), showed that the differences observed were significant ($P < 0{\cdot}001$); further analysis showed that differences between prophase and

Table 4. *Numbers of algae in digestive cells at different stages of the cell cycle, 24 h after feeding, in the gastric region*

	Interphase*	Prophase	Metaphase	Anaphase	Telophase
Number of algae/cell	15·47 ± 0·31	17·56 ± 0·60	19·43 ± 0·56	22·02 ± 0·97	21·49 ± 0·88
Number of observations	331	68	147	44	72
Range	4–31	6–30	8–54	10–39	10–48

Results are from 10 replicate macerations of five gastric regions.
* Interphase cells counted in the same macerations as mitotic stages.

Table 5. *ANOVA test for differences in numbers of algae per cell between cells in prophase, metaphase, anaphase and telophase, 24 h after feeding*

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Stages	783·47	3	261·16	5·97
Residual	14303·73	327	43·74	
Total	15087·20	330		

Table 6. *Numbers of algae in digestive cells at different stages of the cell cycle in regenerating peduncles, 48 h after excision*

	Interphase*	Prophase	Metaphase	Anaphase	Telophase
Number of algae	12·50 ± 0·25	20·77 ± 0·87	19·99 ± 0·50	19·28 ± 0·61	21·06 ± 0·76
Number of observations	360	62	131	46	120
Range	3–31	7–50	3–40	6–32	5–43

Results are from 10 replicate macerations each of five peduncles.
* Counted at time of excision (0 h).

metaphase, and between metaphase and anaphase, were also significant ($P < 0·05$), although there was no significant difference between anaphase and telophase ($P > 0·10$).

Counts of numbers of algae in dividing digestive cells in regenerating peduncles (Table 6), and analysis of variance (Table 7), showed no significant difference between the different stages of mitosis ($P < 0·01$). This agrees with previous work that suggested that first algae and then digestive cells divide in regenerating peduncles (McAuley, 1981*a*, 1982). Since algal division was already complete, there was no increase in numbers of algae as digestive cells proceeded through mitosis. Comparison of numbers of algae in cells at excision with numbers in cells entering prophase 48 h later showed that the size of the algal population had almost doubled. Since symbiotic algae produce four daughter cells at division, the increase observed

Table 7. *ANOVA test for differences in numbers of algae per cell between cells in prophase, metaphase, anaphase and telophase in regenerating peduncles, 48 h after excision*

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio
Stages	140·67	3	46·89	1·15
Residual	14443·73	355	40·69	
Total	14584·40	358		

Table 8. *Variance and coefficient of variation of algal populations before and after digestive cell division*

	Interphase	Telophase	
		Mother cells	Daughter cells
Variance (S)	21·79	68·95	21·25
Coefficient of variation*	37·34	39·43	43·78

*$c.v. = \frac{100S}{\bar{x}}$.

here represents 3·3 algal divisions per cell or about one quarter of the initial population undergoing division prior to host cell mitosis.

When host cell mitosis was stimulated by feeding, multiplication of the algal population began at the onset of host cell division, while during regeneration doubling of the algal population was completed before host cell division began. While the former situation is the normal relationship, the latter experimental situation permits prediction of the way in which algal division is controlled by comparing variance and coefficient of variation of populations of algae in telophase cells (at the end of algal division) and in daughter cells produced by cell division (at the beginning of a new round of replication). Hennis & Birky (1984) described four models of chloroplast replication and their predicted effects of pre-division and post-division variance and coefficient of variation. In hydra digestive cells (Table 8), telophase variance was about 3·25 times that of daughter cells and the coefficient of variation was 0·9 times that of daughter cells. This corresponds with the model described by Hennis & Birky (1984) in which most cells exactly double the numbers of algae before dividing, but cells with numbers at or below a lower threshold double the number of algae twice, while cells with numbers at or above an upper threshold divide without any algal division. Variance and coefficient of variation of populations of telophase daughter cells were similar to those of interphase cells at the beginning of regeneration. This suggests that digestive cells in peduncles contain static populations of algae in which growth has been arrested after a final round of division, as suggested by Douglas & Smith (1984) to explain the lower numbers of algae per cell and the higher mean cell size compared to algae in the more actively dividing gastric region.

DISCUSSION

This paper shows that there is only a partial correlation between the size of a digestive cell and numbers or total volume of the algae it contains; only 50 % or less of the variation in the size of algal populations could be attributed to digestive cell size at interphase, prophase or telophase. Possibly, there may be some effect of host cell size in regulating algal population size when the latter reaches an upper limit, but it is difficult to see how the host cell is able to limit total algal volume per cell, as algal

cell growth (as opposed to cell division) is independent of host cell division and continues, albeit slowly, during extended periods of host starvation (McAuley, 1985*b*). While several workers have shown that numbers of chloroplasts increase in cells of leaf tissue as the average cell size increases (Boason *et al.* 1972; Kameya, 1972; Tsuji *et al.* 1979; Scott & Possingham, 1980; Whatley, 1980; Wild & Wolf, 1980; Olszewska *et al.* 1983), no such relationship appeared to apply between digestive cells and their symbiotic algae.

When hydra were fed, numbers of algae per cell increased as mitosis of host cells proceeded, although there appeared to be a pause in algal division between anaphase and telophase. In excised regenerating peduncles, in which digestive cell mitosis is delayed and algal cell division is completed before that of their host cells (McAuley, 1981*a*, 1982), the average numbers of algae almost, but not quite, doubled in digestive cells that were stimulated to divide. Thus, the increase in algal numbers necessary to compensate for the production of two daughter cells at digestive cell division was almost entirely associated with host cell mitosis. The fact that numbers of algae did not quite double in dividing digestive cells indicates that there may be a small amount of algal division associated with later host cell growth; alternatively, it is possible that even after 48 h algal mitosis was not quite complete. Again these observations rule out an important role in the regulation of numbers of algae by limitation to a certain volume of the host cell.

The most satisfactory model to explain these observations is one in which the proportion of algae dividing as a result of host cell mitosis is greater in digestive cells with few algae than in those with many algae. This type of control of replication was proposed by Hennis & Birky (1984) for division of chloroplasts of the unicellular marine alga *Olisthodiscus*, and variance in populations of algae in telophase mother and daughter cells corresponded to predictions based on this model.

The first paper in this series suggested that algal division was dependent upon host supply of 'division factor', an unknown metabolite derived from host digestion of food (McAuley, 1985*b*), and observations described here are consistent with this. In cells with large numbers of algae, competition for division factor would be more limiting with regard to algal division than in cells with few algae. Since large digestive cells may possess a large pool of division factor, if only because of their greater phagocytic capacity, size of host cells may have an indirect rather than a direct influence on numbers of algae.

Finally, the wide variation in numbers of algae may be explained by two factors. First, since almost all algal division is associated with host cell mitosis, and in fed hydra algal division may not be completed until after host cell telophase, any effect of host cell size on numbers of algae (by determining the proportion that divides) would be manifested in the daughter cells, which may themselves be of unequal size. Second, the way in which algae are distributed between daughter digestive cells at telophase may be important. If algae are distributed equally, then daughter cells would inherit only the variation of the parent population. Conversely, if the distribution tends to be random, as preliminary results have suggested (McAuley,

1982), then a new element of variation would be introduced at each round of cell division. Work is in progress to investigate this problem.

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