

CONTROL OF CELL DIVISION OF THE INTRACELLULAR *CHLORELLA* SYMBIONTS IN GREEN HYDRA

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SUMMARY

Green hydra maintains within its digestive cells a population of symbiotic algae which remains constant in normal culture conditions, although potentially the algae have a much higher growth rate than their animal hosts. Numbers of algae per cell vary along the body column, cells in the gastric region containing more than those of the head or peduncle.

This relationship is disturbed in excised, regenerating peduncles and heads; a transitory increase in algal numbers occurs, the decline of which may be prevented by application of the mitotic inhibitor vinblastine sulphate. No increase is seen in the already high numbers of algae in the gastric regions of regenerating animals.

A close link between host and symbiont mitosis may explain this phenomenon. Non-dividing host cells with a full complement of algae inhibit symbiont mitosis; the inhibition is removed when the host cell is stimulated to divide, as in regenerating peduncles and heads. Algae divide more rapidly than host cells, so a transient increase in algal numbers occurs. During host cell division, algae are parcelled between daughter cells, which reimpose inhibition once the normal population of algae is reached.

There may be no increase in algal numbers in regenerating gastric regions because extensive mitosis already occurs.

The nature of the restriction on algal growth remains obscure, but uncoupling of animal and algal mitosis during regeneration suggests a useful experimental approach to the problem.

INTRODUCTION

The freshwater coelenterate, *Hydra viridissima* (syn: *Chlorohydra viridissima*) maintains actively growing, photosynthetically competent algal symbionts of the genus *Chlorella* within each of its endodermal digestive cells; the algal symbionts may also be cultured independently in a simple mineral medium (Jolley & Smith, 1978). In normal conditions of growth, in the light, the total number of algae per hydra remains stable, and neither partner outgrows the other (Pardy, 1974). Since the rate of growth of algal symbionts in culture is approximately 20 times the maximum observed in host animals (Jolley & Smith, 1978), some mechanism must exist whereby the host animal regulates the growth of its algal symbionts.

Pardy & Muscatine (1973) and Pardy (1974) showed that the average number of algae per digestive cell in the middle, gastric, region of the body column in the Florida strain of green hydra was approximately 50% greater than that in cells of either the head or peduncle regions. Apparently, algal populations are regulated at a higher

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level in the gastric region. Pardy (1974) suggested that this might be due to the higher incidence of mitosis in that region (Campbell, 1967; Park, Ortmeyer & Blankenbaker, 1970; Bisbee, 1973) and that dividing cells contained more algae than non-dividing cells.

Pardy & Heacox (1976) demonstrated that this close regulation was disturbed in regenerating peduncles of the European (English) strain of green hydra. In excised peduncles, numbers of algae per cell temporarily increased, returning to normal by the time the head was regenerated. This temporary increase was prevented by grafting heads onto freshly excised peduncles. It was suggested that the head, known to be responsible for maintenance of polarity and other morphological phenomena in hydra (Burnett, 1966; Webster, 1966; Wolpert, Clarke & Hornbuch, 1972) may be able to influence reproduction of symbiotic algae in the peduncle. Although there are other possible explanations for this phenomenon, the original observation of Pardy and Heacox of a temporary change in numbers of symbionts during regeneration in excised peduncles offers a potentially valuable new avenue for the exploration of mechanisms of symbiont regulation.

This paper describes experiments based on the phenomenon observed by Pardy & Heacox (1976), but which suggest that the head region is unlikely to control algal reproduction in the peduncle. Instead, within an individual digestive cell, there appears to be a close relationship between host cell and symbiont mitosis. The experiments also offer a confirmation of the theory of Pardy (1974) that higher numbers of symbionts in cells in the gastric region may be due to the higher incidence of mitosis in that region than in others.

MATERIALS AND METHODS

Materials

Stock cultures of the European (English) strain of *Hydra viridissima*, originally obtained from Dr L. Muscatine, were maintained in 'M' solution (Muscatine & Lenhoff, 1965) in a Gallenkamp illuminated incubator at 20 °C with a 12-h light/12-h dark photoperiod. Light intensity was 750 lux. Cultures were fed on Monday, Wednesday and Friday with freshly hatched *Artemia salina* nauplii (Loomis & Lenhoff, 1956). All experiments were performed on animals which had not been fed for 24 h.

Estimation of numbers of algae per digestive cell

Preparations of digestive cells were obtained by the method of David (1973). Five experimental organisms were isolated in a drop of macerating fluid (water:glycerol:glacial acetic acid, 13:1:1, v:v) on a glass slide. After 5 min the tissues could be teased apart with dissecting needles, and the resulting suspension of cells was examined using $\times 400$ phase-contrast microscopy. The algae in 150 randomly selected digestive cells were counted (Pardy & Heacox, 1976).

Estimation of mitotic activity

Macerates of the pieces of hydra were allowed to dry on a glass slide and were stained with Mayer's haematoxylin and eosin Y (Grimstone & Skaer, 1972), taken through an ethanol series, and mounted in DPX. In each preparation 400 digestive cells were examined and scored for the presence or absence of mitotic figures.

Inhibition of mitosis

A 10^{-6} M solution of vinblastine sulphate in M solution was used to inhibit animal mitosis. A similar concentration has been shown to inhibit mammalian mitosis (Maio & Schildkraut, 1966). Vinblastine is an anti-neoplastic, anti-mitotic agent known to precipitate tubulin (Cutts, Beer & Noble, 1960). Vinblastine did not cause any abnormalities of regenerating peduncles similar to those observed by Webster (1967) in regenerating colchicine-treated hydra.

RESULTS

Numbers of algae per digestive cell in different regions of hydra

The histograms in Fig. 1 illustrate the differences in distribution of numbers of algae per digestive cell in the 3 regions (Pardy & Muscatine, 1973) of the body column of European hydra. Digestive cells from the head or peduncle contain an average of 15 algae, while those from the gastric region contain 20 algae.

Changes in algal number during regeneration in excised peduncles and heads

Pardy & Heacox (1976) suggested that the temporary rise in numbers of algae per cell during regeneration in excised peduncles was due to removal of the head region, which exerted some influence on algal reproduction. In the following experiments, the effects of excision and regeneration on algal numbers were compared in heads and peduncles.

Heads (tentacles, hypostome and a small collar of tissue beneath) or peduncles of hydra were excised and transferred to M solution in small glass Petri dishes, and maintained in the same conditions as control (starved) hydra. Numbers of algae per digestive cell were counted at 12-h intervals. Fig. 2A,B shows that changes in number of algae per cell followed closely similar paths during regeneration in both peduncles and heads: a rise from about 15 to a peak of 18–19 algae per cell 36 h after excision, followed by a decline to the original level after 72 h. At that time peduncles had grown tentacles, and heads had grown bases and were attached to the substrate. It therefore seems unlikely that the head region controls algal reproduction.

Changes in algal numbers in the gastric region during regeneration of heads or peduncles

Two groups of experimental animals were prepared: in one the peduncle was removed to leave the upper two-thirds of the animal intact, and in the other the head was removed to leave the lower two-thirds intact. At 24-h intervals the gastric region was isolated from animals of each group and the average number of algae per digestive cell was calculated.

Peduncles were regenerated 24 h, and tentacles 48 h, after excision. Throughout the regeneration period, however, there was no discernible change in algal numbers in cells of animals of either group (Fig. 3).

Incidence of mitosis in excised peduncles during regeneration

The experiments described above suggest that the head region does not influence algal reproduction. An alternative explanation of the temporary increase in algal

numbers in peduncles after excision could be that regeneration stimulates both algal and host cell mitosis, but that algal mitosis occurs before that of the host.

The effect of regeneration upon the incidence of host cell mitosis was therefore examined. Regenerating peduncles were macerated and stained with Mayer's haematoxylin and eosin Y 24 h (during increase in algal numbers) and 48 h (during decrease)

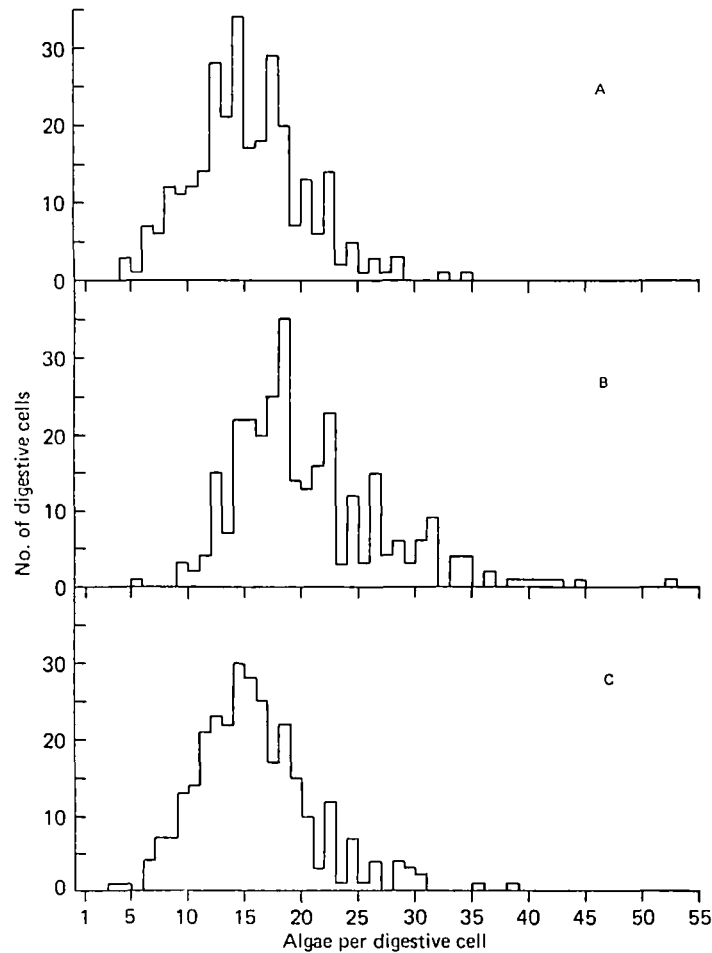


Fig. 1. Distribution according to numbers of algae contained in 300 digestive cells from each of the regions of European hydra at 20 °C. Amalgamation of duplicate counts of 150 cells randomly selected from macerates of 5 pieces of hydra. A, Zone I: tentacles and hypostome; B, Zone II: gastric and budding region; C, Zone III: peduncle and base.

after excision, and mitotic figures were counted in each of 5 groups. Control peduncles were freshly excised from starved animals.

The results (Table 1) show that normally there is a very low incidence of mitosis in the peduncles, while 24 and 48 h after excision there is a significant increase in mitosis ($P < 0.05$) in regenerating peduncles. At 48 h there were significantly more

mitotic figures than at 24 h ($P < 0.05$). These results suggest that digestive cell mitosis increases during regeneration, and that it is higher during the decrease in numbers of algae per cell.

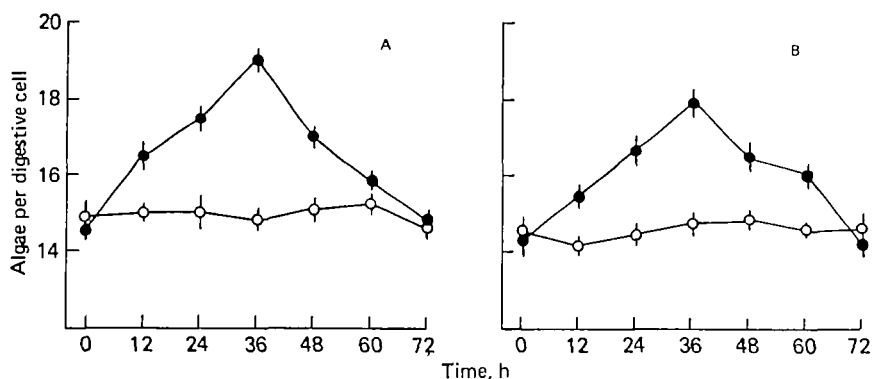


Fig. 2. A, mean number of algae per digestive cell in peduncles of hydra. B, mean number of algae per digestive cell in heads of hydra. Each point is the mean of duplicate experiments in which 150 cells were counted from macerates of 5 pieces. Vertical bars show standard error of mean. ●—●, regenerating, excised pieces; ○—○, control pieces removed from unfed animals at time of counting.

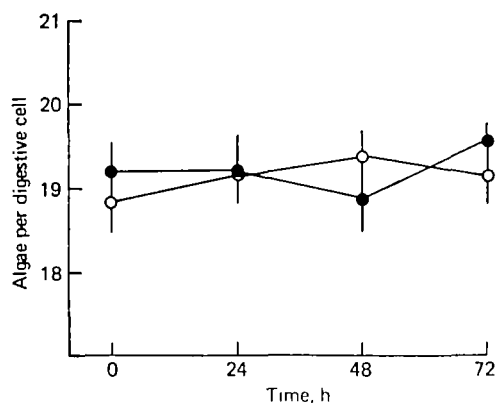


Fig. 3. Mean number of algae per digestive cell in gastric and budding regions of hydra. Each point is the mean of duplicate experiments in which 150 cells were counted from macerates of 5 pieces. Vertical bars show standard error of mean. ●—●, gastric region from which peduncle was amputated; ○—○, gastric region from which head was amputated.

Effects of mitotic inhibitors

Effect of vinblastine sulphate during regeneration. 10^{-6} M vinblastine sulphate was used to confirm that the fall in algal numbers in regenerating peduncles was due to animal cell mitosis. Peduncles were excised and allowed to regenerate normally in M solution for the first 36 h. The usual pattern of increase in algal numbers was followed up to that point (Fig. 4). At 36 h after excision, the regenerating peduncles were transferred to medium containing vinblastine sulphate. There was only a slight

decline in algal numbers subsequent to this, and it was assumed that the treatment had prevented division of animal cells and hence parcelling of algae into daughter cells.

Effects of darkness on regeneration in peduncles. Pardy (1974) demonstrated that continuous darkness specifically inhibited algal growth in green hydra. Animal growth was not affected. This observation was used to provide further evidence for the delay in host cell mitosis until 36 h after excision.

Table 1. *Incidence of mitotic figures in digestive cells in regenerating and control peduncles*

Time after excision, h	Control (n = 2000)	Regenerating
24	6	14
48	2	46

400 digestive cells were scored for the presence or absence of mitotic figures in 5 replicate samples each of 5 peduncles macerated together. Control peduncles were excised from starved hydra at time of counting.

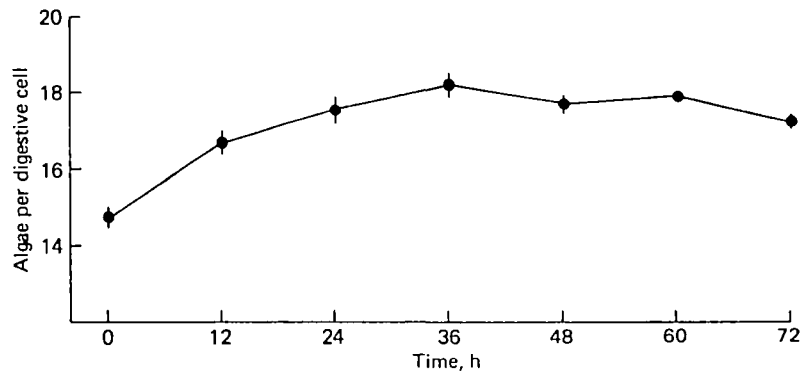


Fig. 4. Mean number of algae per digestive cell in regenerating peduncles transferred to 10^{-6} M vinblastine sulphate at 36 h. Each point is the mean of duplicate experiments in which 150 cells were counted in macerates of 5 pieces. Vertical bars show standard error of mean.

Amputated peduncles were incubated in M solution in dishes wrapped in silver foil. They were exposed to light only when samples were removed for counting. Algal counts were carried out at 12 h-intervals. Controls (peduncles freshly isolated from animals starved in darkness) were counted at 24-h intervals. It was found (Fig. 5) that there was no increase, but a slight decrease, in algal numbers per cell in amputated peduncles, until about 36 h. Thereafter, the decline was more rapid, to a final level that was 75% of control values. Control peduncles showed a slight constant decline over the experimental period, presumably due to dilution of the standing, non-multiplying, crop of algae through normal animal cell division. Amputated peduncles

incubated in darkness in 10^{-6} M vinblastine showed no decline in algal numbers over the experimental period.

Ejection of algae

From evidence presented above, it is postulated that the decline in algal numbers after the initial increase during regeneration was due to animal cell division. An alternative to this is that excess algae could be ejected by the host cell. Although ejection of algae from digestive cells cannot be detected under normal conditions (Muscatine & Pool, 1979; McAuley, 1980), it may occur if hydra are subjected to

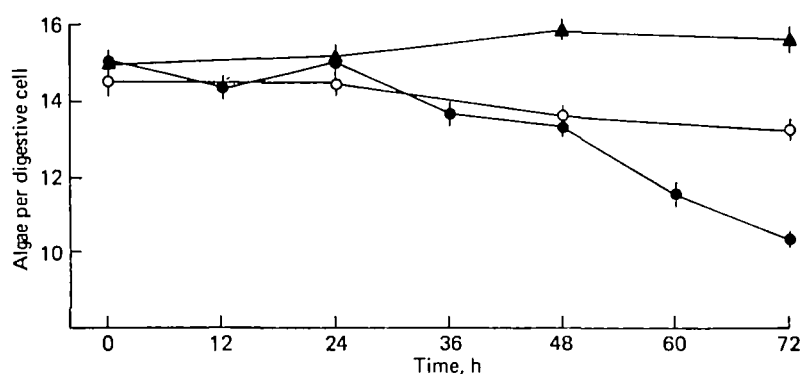


Fig. 5. Mean number of algae per digestive cell in peduncles in darkness. Each point is the mean of duplicate experiments in which 150 digestive cells were counted in macerates of 5 peduncles. Vertical bars show standard error of mean. ●—●, regenerating, excised peduncles; ○—○, control peduncles removed at time of counting from unfed animals; ▲—▲, excised peduncles incubated in medium containing 10^{-6} M vinblastine sulphate.

Table 2. Position of algae in digestive cells of regenerating and control peduncles after 48 hours

	No. of algae above nucleus	No. of algae below nucleus	Total no. of algae	% of algae above nucleus
Regenerating	345	2705	3050	11.3
Control	332	2486	2818	11.8

Figures are the amalgamation of measurements of 180 digestive cells, 10 from each of 18 peduncles. Measurements were made 48 h after excision in the case of regenerating peduncles; controls were excised at time of counting from starved hydra.

stresses such as irradiation at very high light intensities (Pardy, 1976; Steele & Smith, unpublished), or incubation in glycerol or the antibiotic trimethoprim (McAuley, 1980). Ejection is manifested by extrusion of cohesive pellets of algae, and by movement of algae in digestive cells from their normal position at the base towards the apex, above the animal cell nucleus.

Careful examination of regenerating peduncles revealed no extrusion of algal pellets. Measurement of the position of algae in 180 randomly selected digestive cells

of regenerating peduncles 48 h after excision showed no increase in numbers of algae located above the nucleus – usually a precise measure of ejection (McAuley, 1980) – as compared with non-regenerating controls (Table 2).

DISCUSSION

Green hydra exercises strict control over the number and reproduction of its intracellular algal symbionts. This control appears to be exercised at the level of the host digestive cell. Contrary to the suggestion of Pardy & Heacox (1976), the head appears to have no influence on algal reproduction in other parts of the body column, since changes in algal number followed exactly the same course in excised peduncles as excised heads (Fig. 2 A, B).

The experiments described here suggest that there is a close link between host cell and symbiont mitosis. In non-dividing host cells containing a full complement of algae there is presumably some factor which inhibits algal mitosis. However, when the host cell is stimulated to divide (as in regenerating heads or peduncles) the restriction on algal mitosis is removed. Algal mitosis occurs more rapidly than host cell mitosis, so that there is a period when host cells contain increased numbers of algae. After the host cell has divided, the restriction on algal mitosis is reimposed when the full complement of algae is reached. Possibly, the rapid removal of the restriction of algal mitosis is of advantage to the host in that daughter cells will already contain more than half the normal complement of algal symbionts. Presumably, not all algae divide at the onset of regeneration. *Chlorella* symbionts divide into 4 auto-spores (Oschmann, 1967), so that division of total algal population would result in a 300% increase in numbers of algae per digestive cell. The 25% increases observed here are commensurate with 1 in 6 algal cells dividing in regenerating heads or peduncles.

That mitosis of symbionts and host cells occurs at different times was emphasized by the use of the anti-mitotic drug vinblastine sulphate, and by specifically inhibiting algal mitosis through incubating excised peduncles in constant darkness. When vinblastine was applied at the peak of the temporary rise in algal numbers, host cell division was presumably blocked and there was no rapid decline in numbers of algae per cell. Incubation of peduncles in constant darkness showed that algal numbers declined after 36 h even if there was no prior increase.

These experiments also contribute towards the explanation of Pardy (1974) that numbers of algae per cell are normally higher in the gastric region of the hydra body column because animal cell mitosis is greater there than elsewhere. Several workers have shown that mitosis is unnecessary during regeneration of missing heads or peduncles by the body column (Park, 1958; Webster, 1967; Corff & Burnett, 1969; Hicklin & Wolpert, 1973). Thus, the absence of any increase in numbers of algae during regeneration in the gastric region may be because sufficient reserves of tissue are already present and so no increase in host cell mitosis occurs. Excised heads and peduncles have much smaller reserves and depend on mitosis to regenerate missing parts.

The question of what restricts the division of algae once a certain level of infection is reached remains unanswered. The original observation of Pardy & Heacox (1976), that symbiont and host cell mitosis become temporarily uncoupled during regeneration, offers a useful experimental approach.

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