TEMPORAL RELATIONSHIPS OF HOST CELL AND ALGAL MITOSIS IN THE GREEN HYDRA SYMBIOSIS

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SUMMARY

In fed hydra or excised regenerating peduncles there are increases in the mitotic indices of both digestive cells and the symbiotic algae that reside within them. Conversely, algal and digestive cell mitotic indices decrease in starved hydra. The temporal relationships of algal and host cell division differ in fed hydra and regenerating peduncles. After feeding, algal and digestive cell mitotic indices both reach a peak at about the same time; during regeneration, first the algae and then the digestive cells divide. Thus, mitotic digestive cells in regenerating peduncles contain more algae than those in gastric regions of fed hydra. However, in both cases mitotic digestive cells contain more algae than non-mitotic cells. The algae appear to be partitioned at random between daughter digestive cells at telephase.

It is suggested that the division of the symbiotic algae is closely related to that of the digestive cells in which they are maintained. Mitosis of algae is stimulated by host cell mitosis, but in non-dividing cells algal mitosis is restricted. Possible mechanisms by which the host digestive cells could restrict algal division are discussed.

INTRODUCTION

The stability of a mutualistic symbiosis depends in part upon the relationship of the growth rates of the two partners. Provided these are regulated to similar levels, the symbiosis will persist (Droop, 1963). At present, the mechanisms that regulate growth rates and sizes of symbiont populations are poorly understood.

Unicellular symbiotic Chlorella reside within individual vacuoles in the endodermal digestive cells of green hydra. The growth rates of the hydra and its symbiotic algae have been shown to be identical, and numbers of algae per cell remain constant in constant conditions, although environmental changes such as photoperiod or temperature may change the relationship (Pardy & Muscatine, 1973; Pardy, 1974; McAuley, 1980). Further, although different strains of green hydra contain different numbers of differently sized algae, the relationship between host size and the volume of algae contained is similar (McAuley, 1981a). This applies also to different strains of algae artificially symbiotic with the ‘European’ strain of green hydra (Mews & Smith, 1982). Since, under normal conditions, the size of the algal population is proportional to the size of the host, growth of the algae must be dependent upon host growth.

Pardy (1974) suggested that division of the algal symbionts may be linked to division
of the host cell. Pardy & Heacox (1976) observed that in excised regenerating peduncles numbers of algae increased and then declined; McAuley (1981b) showed that host cell mitosis was highest during the decline in numbers of algae per cell and that the mitotic inhibitor vinblastine sulphate, applied at the beginning of the decline in algal numbers, prevented it, McAuley (1981b) suggested that there was a close link between host and symbiont mitosis in regenerating peduncles. Thus, non-dividing host cells with a full complement of algae inhibited algal mitosis, but this inhibition was removed when the host cell was stimulated to divide. The algae divided more rapidly, causing the transitory increase in numbers of algae per digestive cell; when the host cells divided, the daughter cells parcelled out the algae and reimposed the inhibition once the normal population level had been reached.

Whereas the above observations support the idea of a close link between host and symbiont mitosis, the temporal relationships have not yet been investigated. In experiments described in this paper, digestive cell and algal mitotic indices were measured after feeding and during regeneration to define their relationships. The distribution of algae between daughter cells was also examined.

**MATERIALS AND METHODS**

**Experimental organisms**

Stock cultures of the 'European' strain of *Hydra viridissima* (syn: *Hydra viridis, Chlorohydra viridissima*) were maintained in 'M' solution (Muscatine & Lenhoff, 1965) at 18 °C in an illuminated incubator (irradiance 1.9 × 10⁻⁶ Einsteins m⁻² s⁻¹; 12 h light/12 h dark photoperiod). Cultures were fed (Monday, Wednesday and Friday) with freshly hatched nauplii of *Artemia salina* (Loomis & Lenhoff, 1956). Hydra or portions of hydra treated experimentally were maintained in the same conditions as stock cultures. All hydra used experimentally bore a single bud and unless otherwise stated had been starved for 24 h.

**Estimation of mitotic indices**

Mitotic figures of digestive cells were visualized by using the DNA-specific stain 4',6-diamidino-2-phenylindole (DAPI) (Lin, Comings & Alfi, 1977; Kapuscinski & Stoczylas, 1978; Brunk, Jones & James, 1979; Muscatine & Neckelmann, 1981). Five gastric regions or peduncles were isolated on a glass slide in a drop of maceration fluid (David, 1973) containing 5 μg/ml DAPI. After 10 min the pieces of hydra were teased apart, the cell suspension covered with a coverslip, and stored at 4 °C until examined. Overnight storage allowed cells to flatten out so that chromosomal figures could be more easily identified and numbers of algae could be counted accurately. Macerations were examined by × 450 epifluorescence.

Algal mitosis was measured by homogenising five pieces of hydra in a small volume of 'M' solution in a glass microtissue homogenizer and examining the resultant suspension of algae by × 640 light microscopy. Numbers of tetrads (algae dividing into four autospores, stages L4 and Dn) were used as an index of algal cell division (Tamiya, 1963).

**RESULTS**

**Effect of feeding and starvation on host cell and algal mitosis**

Feeding of hydra has been shown to cause an increase in the mitotic index of digestive cells, with a peak 10–12 h after feeding (David & Campbell, 1972; Muscatine & Neckelmann, 1981), presumably as a response to the input of nutrients derived from
digestion of prey. To evaluate the division response of the symbiotic algae to feeding, algal and digestive cell mitotic indices were measured 24 h after feeding hydra that has been starved for 72 h. Starvation reduces digestive cell mitosis (Muscatine & Neckelmann, 1981), so that the effect of feeding would be more pronounced. Table 1 shows that both algal and digestive cell mitosis was higher in fed hydra than in unfed controls, suggesting at least an indirect link between host cell and algae division.

### Table 1. Effect of feeding on digestive cell and algal mitosis

<table>
<thead>
<tr>
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<th>No. mitotic cells/1000</th>
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<tbody>
<tr>
<td></td>
<td>Fed</td>
</tr>
<tr>
<td>Digestive cells</td>
<td>9.8 ± 0.6</td>
</tr>
<tr>
<td>Algae</td>
<td>10.8 ± 2.6</td>
</tr>
</tbody>
</table>

Fed hydra were starved for 72 h, fed, and sampled 24 h later. Starved hydra were not fed for 96 h. Figures are means ± S.E.M. of five counts of mitotic cells per 1000 digestive cells in macerates or algae in homogenates of excised gastric regions of five hydra.

Fig. 1. Mitotic indices of digestive cells and algae in gastric regions after feeding. A, Digestive cells; B, algae. Bar indicates photoperiod (open, light; solid, dark). Each point is the mean of two independent experiments in which numbers of dividing forms were counted in 1000 cells.

The response of host digestive cells and symbiotic algae in the 24 h following feeding was examined in more detail by counting numbers of dividing algae and mitotic digestive cells at 3 h intervals following addition of *Artemia nauplii*. Fig. 1 shows that both algal and digestive cell division increased after feeding; the peak of division in both was reached in about 12 h and declined thereafter. When hydra are fed, both host cells and symbionts respond by division.
While feeding hydra causes an increase in mitosis of digestive cells and algae, starvation causes a decrease. Fig. 2 shows the mitotic indices of both at 24 h intervals after a single feeding. During starvation, the mitotic indices of algae and digestive cells fell rapidly to a low level, suggesting that an input of nutrients is necessary so that both may divide.

Digestive cell and algal mitosis during regeneration in excised peduncles

The experiments described above do not show whether the increase in algal division observed after feeding hydra was due to a direct response to an increase in available nutrients, or to a response to digestive cell mitosis. During regeneration in
excised peduncles, where no feeding takes place, algal numbers per cell showed a transitory increase (Pardy & Heacox, 1976; McAuley, 1981b), and it was suggested that there was a direct link between algal and digestive cell mitosis (McAuley, 1981b).

In the following experiment, the mitotic indices of digestive cells and algae were measured at 12 h intervals in excised peduncles. The results are shown in Fig. 3. The algae appeared to divide within the first 36 h after excision, while digestive cell mitosis did not increase much above levels in control peduncles (isolated from starved hydra at time of counting) until 36-48 h after excision. These results are commensurate with the experiments of Pardy & Heacox (1976) and McAuley (1981b), in which it was observed that numbers of algae per cell showed a transitory increase. They suggest that algal division may occur without the addition of nutrients to the symbiosis (as in

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<thead>
<tr>
<th></th>
<th>Mitotic</th>
<th>Non-mitotic</th>
<th>P</th>
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<tr>
<td>Fed*, gastric regions</td>
<td>21.18 ± 0.60</td>
<td>17.84 ± 0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Regenerating† peduncles</td>
<td>25.47 ± 0.87</td>
<td>17.61 ± 0.63</td>
<td>&lt;0.001</td>
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Numbers of algae were determined in 20 mitotic and 20 non-mitotic digestive cells in each of five macerates of five gastric regions or peduncles. Figures are means ± s.e.m. P is the probability that numbers of algae in mitotic and non-mitotic digestive cells are similar.

* 24 h after feeding hydra previously starved for 72 h.
† 48 h after peduncles excised.

Numbers of algae in mitotic digestive cells

Numbers of algae per cell were counted in mitotic and non-mitotic digestive cells in macerates of either gastric regions of fed hydra or of regenerating peduncles. The results are given in Table 2. In both cases, mitotic digestive cells contained more algae than non-mitotic cells. The difference between mitotic and non-mitotic cells was greater in regenerating peduncles. This may be explained by the results shown in Figs. 1 and 3. In regenerating peduncles, algal mitosis is completed before that of the digestive cells begins, so that if algal and host cell division are closely linked, dividing digestive cells would contain the maximum number of algae. In fed hydra, the onset of algal and digestive cell mitosis is more closely related, so that digestive cells divide before algal division is complete.

The observation that mitotic digestive cells contain higher numbers of algae than non-mitotic digestive cells suggests a close link between host and algal mitosis; that algae divide in host cells entering mitosis.
It should be noted that numbers of algae in non-mitotic digestive cells in regenerating peduncles were higher than the normal value (approx. 14-15; McAuley, 1981b). This is presumably because of the presence of digestive cells in which the algae had divided but cell mitosis had not yet begun.

**Partition of algae between daughter digestive cells**

In digestive cells at teleophase, the daughter cells were often sufficiently separated so that numbers of algae could be counted in each. Table 3 shows the differences in distribution of algae amongst daughter digestive cells from gastric regions of fed hydra and from regenerating peduncles. Paired *t*-tests showed that differences observed varied significantly from the differences of zero expected if the algae were distributed equally. It is suggested that the population of algae in a digestive cell at teleophase are partitioned between daughter cells at random.

<table>
<thead>
<tr>
<th>Table 3. Differences in distribution of algae in daughter digestive cells at teleophase</th>
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<tbody>
<tr>
<td>No. teleophase cells</td>
</tr>
<tr>
<td>----------------------</td>
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<tr>
<td>Fed, gastric regions</td>
</tr>
<tr>
<td>Regenerating peduncles</td>
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* Probability that observed difference in distribution of algae is similar to the expected difference (= 0) if algae were distributed equally (paired *t*-test).

**DISCUSSION**

The division of symbiotic *Chlorella* appears to depend upon some function of the digestive cells in which they are located. Observations described here suggest that when hydra are fed, mitosis of both digestive cells and algae is stimulated. Conversely, mitotic indices of digestive cells and algae fall to low levels during starvation. Mitosis of digestive cells and algae also increases during regeneration in excised peduncles, confirming previous experiments (McAuley, 1981b).

However, temporal relationships of host cell and symbiont mitosis differ in fed hydra and regenerating peduncles. After feeding, algal and host cell division increases in an approximately parallel manner, both reaching a peak after about 12 h. During regeneration, algal mitosis precedes that of digestive cells, explaining the transitory increase in numbers of algae per digestive cell observed by Pardy & Heacox (1976) and McAuley (1981b). Although mitosis of both algae and digestive cells is stimulated in regenerating peduncles, that of the latter appears to be delayed. Bode (1973) has suggested that digestive cells in the peduncle may have differentiated and hence lost their ability to divide; the delay between excision and digestive cell mitosis observed here may be due to the need for digestive cells in the peduncle to resynthesize the capacity for division.

The difference in the relationship between host cell and algal mitosis in fed hydra and regenerating peduncles is confirmed by comparing numbers of algae in mitotic
Relationship of host and symbiont mitosis

digestive cells. As would be expected, those from regenerating peduncles contain more algae than those from gastric regions of fed hydra, since in regenerating peduncles algal mitosis is virtually complete before that of digestive cells begins. However, in both fed hydra and regenerating peduncles, mitotic digestive cells contain more algae than non-mitotic cells, suggesting that the increases in algal mitosis are closely related to mitosis of the host cells.

The experiments described here agree with the hypothesis that algal mitosis is inhibited in non-mitotic digestive cells, but at digestive cell mitosis the inhibition is removed and the algae divide (McAuley, 1981b). It should be noted that the separation of algal and digestive cell mitosis observed in regenerating peduncles is probably a special case. Normally, as observed in fed hydra, algal and digestive cell mitosis occur at almost the same time. Presumably, algal division continues in daughter cells until limits are reached and the inhibition is reimposed.

Since symbiotic algae are autotrophs with a potentially higher division rate than their animal hosts, whose growth at least in part depends upon heterotrophic nutrition, control of algal proliferation by the host is important. The mechanism by which hydra regulate their population of symbiotic algae, by inhibiting algal division except when the host cell divides, has parallels in other intracellular symbioses. When the protozoan Paulinella chromatophora, which contains two cyanellae, divides, each daughter cell receives a cyanella, which in turn divides to return the number to two per cell (Pascher, 1929a,b). Division of the large bacterial symbionts of Pelomyxa palustris is at a maximum before division of the host cell (Whatley, John & Whatley, 1979). In the Paramecium bursaria/Chlorella symbiosis, Weis (1977) showed that the symbiotic algae appeared to divide synchronously within the host cell, and suggested that this was the basis for the mechanism by which host and symbiont growth rates were equalized. However, in symbioses between marine invertebrates and unicellular algae ejection, perhaps coupled with digestion, appears to be the mode of regulation (for a review, see Trench, 1979). Ejection of either healthy or moribund algae does not appear to occur in green hydra cultured in normal conditions (Muscatine & Pool, 1979; McAuley, 1981c).

The mechanism by which digestive cells control the division of the symbiotic algae remains to be elucidated. Pardy (1982) suggested that inhibition of algal growth may be applied at any stage in the life-cycle, since there was no difference in the distribution of algal cell size in populations from either fed or starved hydra (in which mitosis is much lower). Potentially, there are three mechanisms of control. The host cell may secrete an inhibitor of algal growth except during mitosis; or the algae may themselves produce a density-dependent inhibitor, which may be diluted by host cell expansion at mitosis. Finally, the host may inhibit algal growth by controlling the flow of nutrients to the algae (Muscatine & Pool, 1979). Cook (1972, 1976) and Thorington & Margulis (1981) have shown that nutrients are translocated from the host and incorporated into the algae. Pool (1976) and Muscatine & Neckelmann (1981) showed that supplement of 'M' solution with mineral nutrients caused increases in numbers of algae per digestive cell; addition of a combination of nutrients and EDTA caused overgrowth by the algae and death of the hydra (Muscatine & Neckelmann, 1981).
During asexual reproduction of the hydra, the algal symbionts are inherited intracellularly through cell mitosis. Since the algae appear to be distributed at random between daughter digestive cells, phasing of algal and digestive cell mitosis would ensure a maximum number of algae at host cell division and so aid the perpetuation of the symbiosis.

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REFERENCES


Relationship of host and symbiont mitosis


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