COMPETITION BETWEEN CHLORELLAE IN CHIMERIC INFECTIONS OF HYDRA VIRIDIS: THE EVOLUTION OF A STABLE SYMBIOSIS

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SUMMARY

Aposymbiotic polyps of Hydra viridis were infected with one or two of the following strains of Chlorella: the native strain obtained from green H. viridis, and the originally non-symbiotic strains Fs and 211/8p cultured in vitro. Larvae of Artemia served as infecting vectors.

Chimeric infections were obtained with two different Chlorella strains cohabiting in the same cells and polyps. In time, the chimeric infections disappeared and mixed populations of Hydra were formed with different strains of Chlorella in different polyps.

We suggest that the Chlorella/Hydra symbiosis was initiated originally by an infection of preadapted hydra by preadapted chlorellae. Through intracellular interalgal competition and competition between dissimilar infected cells and polyps, the present-day stable symbiosis has evolved.

INTRODUCTION

The green fresh-water Hydra viridis hosts in its digestive cells symbiotic unicellular algae of the genus Chlorella. Morphological and physiological aspects of this symbiosis have been studied (Park, Greenblatt, Mattern & Merril, 1967; Oshman, 1967; Pardy, 1976; Cantor & Rahat, 1982; Rahat & Reich, 1983), but only speculations could be made as to the process by which this symbiosis originated in nature (Smith, 1980; Cook, 1981).

Recently, we have shown that aposymbiotic polyps of H. viridis can be infected with several strains of free-living chlorellae that are apparently preadapted to live in a nutrient rich environment (Rahat & Reich, 1984, 1985) such as the cell of a hydra (Cook, 1972; Thorington & Margulis, 1981).

Thus we obtained a model to imitate nature and in which to investigate experimentally the formation of a new symbiosis through infection of aposymbiotic specimens of H. viridis with different strains of Chlorella.

In this study we present the first report of a chimeric infection in which chlorellae of two different strains cohabit in the same polyp and cell.

We conclude that interalgal competition in the cells of infected H. viridis, competition between dissimilar infected cells in the hydra, and competition between polyps hosting different chlorellae determine which strain remains as the sole symbiont in H. viridis.

Key words: Chlorella, Hydra viridis, symbiosis.
MATERIALS AND METHODS

Stock cultures

Swiss symbiotic *H. viridis* (Ssh), aposymbionts (Sah) derived from the same strain (Rahat, Zeldes & Reich, 1979) and a non-symbiotic brown hydra were used in all our experiments. Three strains of *Chlorella* were used, the originally non-symbiotic Fs and 211/8p grown in vitro (Rahat & Reich, 1985), and the native Ss (Swiss symbiont) obtained by homogenization from Ssh.

Strain 211/8p was chosen as it differs in form from the other two strains and can be distinguished from them even when inhabiting the same cell (Fig. 1).

Hydra and chlorellae were cultured as described before (Rahat & Reich, 1985).

Infection of hydra with chlorellae

Larvae of *Artemia* sp. fed with chlorellae were used as vectors to infect the hydra (Rahat & Reich, 1984). For double infections, e.g. Fs and 211/8p, the respective chlorellae were fed separately to 4 to 5-day-old *Artemia*. The hydra were then offered larvae containing the respective chlorellae (two to three larvae of each).

In this manner we infected Ssh, Sah and hydra already infected by Fs (SFsh) or 211/8p (S211/8ph).

For detailed examination of infection, the hydra were macerated (David, 1973) and the occurrence of the different strains of chlorellae was determined using interference and fluorescence microscopy.

RESULTS

Irregular and uncontrolled infections

As we reported for Fs (Rahat & Reich, 1984), the number of 211/8p chlorellae in cells of hydra and their distribution along the polyps were inconsistent and fluctuated with time. In the same polyp some cells contained up to 30 chlorellae while adjacent cells contained a few only or none at all. Some polyps were completely green while others had irregular patches, and there was no consistency in the proportions of more- and less-infected hydra in a given population.

The 211/8p chlorellae, like Fs, reproduced rapidly in the cells of hydra and surplus algae were continuously expelled from the cells into the coelenteron.

No constant quantitative parameters, e.g. number of chlorellae per hydra cell or polyp, could thus be determined in hydra infected with 211/8p.

All chlorellae infecting brown hydra were eliminated in 1–2 days.

Chimeric infections in cells and polyps

Following double infections, i.e. Ss + 211/8p and Fs + 211/8p, chimeric infections were formed (Fig. 1); chlorellae of two different strains being present in the same cells, and dissimilar infected cells comprising the same polyp (Table 1). Mixed populations resulted, i.e. some hydra contained Ss or Fs and others 211/8p.

Fig. 1. Chimeric infections in cells of macerated *H. viridis*, polyps infected with one or two different strains of *Chlorella* sp. A. Cell with native (Ss) symbionts. B. Two adjacent cells from the same hydra, containing different chlorellae. C. Two different chlorellae in the same cell. D. Cell containing Fs chlorellae at its apex. E. Two different chlorellae in the same cell. F. Cell containing 211/8p chlorellae. Scale, 10 μm between bars. (Note different magnification for c.)
Free-living chlorellae in H. viridis

Fig. 1
Table 1. *Competition of chimeric symbioses in H. viridis*

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Type of host hydra*</th>
<th>Digestive cells free of chlorellae†</th>
<th>Strains of Chlorella sp. used for infection</th>
<th>Quantitative estimation of the different strains of chlorellae in cells of macerated hydra 2–3 weeks after infection‡</th>
<th>Resulting symbioses 2–3 months after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sah</td>
<td>All</td>
<td>Ss, Fs, 211/8p</td>
<td>Ss&gt;&gt;&gt;211/8p&gt;&gt;Ss; 211/8p</td>
<td>Ssh</td>
</tr>
<tr>
<td>2</td>
<td>Sah</td>
<td>All</td>
<td>+, Fs</td>
<td>Fs&gt;&gt;&gt;211/8p</td>
<td>SFsh</td>
</tr>
<tr>
<td>3</td>
<td>Ssh</td>
<td>None</td>
<td>+</td>
<td>Ss only</td>
<td>Ssh</td>
</tr>
<tr>
<td>4</td>
<td>S211/8ph</td>
<td>Some</td>
<td>+</td>
<td>Ss&gt;&gt;&gt;211/8p&gt;&gt;Ss; 211/8p</td>
<td>Ssh</td>
</tr>
<tr>
<td>5</td>
<td>S211/8ph</td>
<td>Some</td>
<td>+</td>
<td>211/8p&gt;&gt;Fs&gt;&gt;211/8p; Fs</td>
<td>S211/8p&gt;&gt;&gt;Fs§</td>
</tr>
<tr>
<td>6</td>
<td>SFsh</td>
<td>Some</td>
<td>+</td>
<td>Fs&gt;&gt;&gt;211/8p&gt;&gt;Fs; 211/8p</td>
<td>SFsh</td>
</tr>
</tbody>
</table>

* Sah, aposymbiotic hydra of the Swiss strain. Sah, Swiss symbiotic hydra containing the native symbionts, Ss. S211/8ph, Sah infected with 211/8p and containing this strain only. SFsh, Sah infected with Fs and containing this strain only.
† In Sah all digestive cells are available for algal infections. In Ssh all digestive cells are occupied by the native Ss symbionts. In hydra containing Fs or 211/8p, only some of the cells are infected, and many digestive cells are still available for new infections.
‡ Ss>>>211/8p>>Ss; 211/8p: the majority of the cells contain Ss only, some contain 211/8p, and a few contain chlorellae of both strains.
§ S211/8p>>>Fs: hydra hosting both 211/8p and Fs, the former being by far more abundant than the latter.
Competition and preservation of territory

In time (weeks or months) the chimeric infections disappeared, first from the cells and then from the polyp, and homogeneously infected cells were predominant among the hydra. Similarly in mixed populations, in a short time, only one strain could be found in each hydra (Table 1).

When Sah was infected with Ss or Fs together with 211/8p, the latter strain eventually disappeared and Ssh or SFsh populations were obtained (Table 1, exp. 1, 2). When hydra already containing a different strain were infected with Sa or Fs chlorellae (exp. 3, 5, 6), the 'settled' chlorellae apparently had some advantage and maintained their territory (McAuley & Smith, 1982). Ss, however, seemed to be more successful and they displaced 211/8p from their intracellular habitat (exp. 4).

Discussion

The inconsistent number of algal symbionts per cell, and the irregular distribution pattern of the 211/8p and Fs infections in hydra, as compared to that of the native Ss symbiotic chlorellae, probably indicate the absence of the long period of coadaptation that the latter had in Ssh. However, both strains formed stable symbioses with H. viridis. No symbioses were formed between brown hydra and these strains, or with five other strains that do infect H. viridis (Rahat & Reich, 1985).

We must thus assume that like the preadaptation required of the chlorellae to enable them to live in hydra, a preadaptation is also required of the hydra. We have no information as to what these preadaptations might be.

The digestive cells of hydra were found to be open to double infections (Fig. 1), being host to different chlorellae for weeks. As in any abiotic habitat, these chlorellae probably also competed for the intracellular habitat. We do not know yet what characteristics enabled one to displace the other (Table 1).

Hydra are known to replace their cells every 2–3 weeks (Campbell, 1967). Thus, when different digestive cells of the same-polyp were infected, respectively, with different strains of chlorellae, the strain that enhanced proliferation of its host cells would apparently soon occupy the whole polyp.

From former studies we know that hydra containing various 'new' symbionts all have a lower budding rate than Ss-containing Ssh, and some have less buds than the others. In a population of dissimilar infected hydra the effect of the respective algal symbionts on budding would certainly affect the long-time survival of a given infection in that population.

We conclude that competition between algae invading a cell of hydra, towards the establishment of a stable symbiosis in the host species, occurs at the intracellular-interalgal, cellular and polyp levels. We should distinguish between an infection formed in a cell of hydra that lasts the life of that cell, a symbiosis in a polyp that lasts the life of that polyp, and a perpetuating symbiosis in the population of a given species of hydra. It is only the last symbionts that would in time coevolve with the host.

On the basis of our results we may now reconstruct the formation and evolution of
the present-day stable Clorella/Hydra symbiosis as it might have happened in nature. Preying on filter-feeding crustaceans, the digestive cells of hydra became infected with several strains of free-living algae. Some strains of Chlorella, preadapted to reproduce in a nutrient-rich environment, survived in cells of preadapted hydra. Through interalgal competition inside these cells, competition between cells of the hydra containing different chlorellae, and competition between differently infected polyps, one strain of chlorellae survived with its host species. In time, through coevolution, the present-day stable symbiosis that is obligatory for the chlorella was formed. Further currently occurring 'foreign' infections are constantly eliminated from the hydra, in which the settled chlorellae preserve their territory.

REFERENCES


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