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Specific Problems of Cell Recognition and Perialgal Vacuole Formation in the *Paramecium bursaria* Endosymbiosis

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Abstract

The sequence of events leading to a permanent symbiotic association between endosymbiotic *Chlorella* sp. and *Paramecium bursaria* as well as information on the formation of perialgal vacuoles (PV) in *P. bursaria* are presented. Uptake of cells of the exosymbiotic *Chlorella* strain by *P. bursaria* always results in the formation of several algae bearing vacuoles from which single-cell-vacuoles (SCV) are liberated during the condensation phase of normal digestive vacuole formation. We suggest that the transformation of SCVs into PVs requires the fusion of SCVs with specific vesicles and/or incorporation of so far unknown membrane material in order to prevent fusion with primary lysosomes. The amount of this specific material might be a limiting factor for PV formation during the establishment of the endosymbiotic entity between *Chlorella* sp. and *Paramecium bursaria*.

Keywords: *Paramecium bursaria*, symbiotic *Chlorella* sp., perialgal vacuoles, single-cell-vacuoles

Abbreviations: DV = digestive vacuole, PV = perialgal vacuole, SCV = single-cell-vacuole

1. Introduction

The establishment of a permanent association and a mutual relationship between endosymbiont and host are prominent features of endosymbiotic associations. In the case of the green *Paramecium bursaria*, this relationship has been established during the course of evolution. So far there are no reports of an endosymbiotic strain of *Chlorella* sp. or an aposymbiotic strain of *Paramecium bursaria* occurring in nature. Apparently both partners have undergone a period of coevolution. During this time they have acquired special features necessary for permanent coexistence. Under laboratory conditions, however, it is possible to isolate the two partners and to cultivate them separately (Pringsheim, 1928; Reisser, 1975, 1976a, 1976b; Wichterman, 1943). When such algae-free *P. bursaria* are incubated with cells of green algae other than the original symbiotic *Chlorella* strain, the algae are usually ingested in DVs, which undergo cyclosis. Only cells from very few strains of free-living *Chlorella* species are not immediately digested but are enclosed in single-cell-vacuoles, where they stay alive for a certain time. However, these SCVs turn into DVs under conditions of limited food supply for the *Paramecium* cells (Niess et al., 1982). Such a transformation never occurs in the true endosymbiotic system. Most cells of the symbiotic *Chlorella* strain are digested when they are incubated with the ciliate. However, some of them escape this fate and get separated into a special type of vacuole, the perialgal vacuole (PV), which protects the algae against the action of lytic enzymes (Bomford, 1965; Hirshon, 1969; Karakashian and Karakashian, 1965, 1973; Karakashian and Rudzinska, 1981; Reisser, 1975, 1976a, 1976b; Siegel, 1960; Weis, 1978). PVs differ from DVs not only in size and shape, but also in the density of their intramembraneous particles (Meier et al., 1980, 1984; Reisser et al., 1985), reflecting specific features of this type of vacuole. These observations raise the following questions: (a) What is the origin of the PV? (b) Where is the process located which recognizes the cells of the symbiotic *Chlorella* strain as suitable for endosymbiosis (recognition process)? (c) When does the decision about the fate of an ingested individual algal cell — digestion or enclosure into a PV — take place? It was the aim of this study of answer these questions and to characterize the sequence of events resulting in the formation of a PV during the establishment of a permanent *P. bursaria* endosymbiosis.

2. Materials and Methods

The algae-free strain of *P. bursaria* (Reisser, 1976a) used in the following experiments was cultivated in a bacterized lettuce medium at 22° C. The symbiotic *Chlorella* sp. strain was grown exosymbiotically in axenic mass culture according to Reisser (1975). For infection experiments, aposymbiotic *P. bursaria* were starved in exhausted lettuce medium for 7 days. At the 8th day the ciliates were harvested by gentle centrifugation (IEC HN-S centrifuge, DAMON, England, 1000 rpm, 40 sec), washed once with sterile lettuce medium and concentrated in small aliquots. The infection experiments were started by adding the algae to the *Paramecium* suspension to a final concentration of 10^9 algae/ml and 10^4 paramecia/ml. The paramecia were isolated after various incubation periods and separated from adhering algae by passing individual ciliates through 4 drops of sterile lettuce medium. In order to determine the number of freshly ingested algal cells in each experiment, some of the ciliates were squashed immediately after incubation with the algae and subsequent washing. The algal cells released from these ciliates were counted by light microscopy. The other paramecia were incubated in a sterile lettuce medium at 22° C under a light/dark regime of 14 hr light and 10 hr dark (Philips fluorescence tubes TL 40@/32 and TL 40W-1/55, 14.9 $W.m^{-2}$). At certain time intervals (stated in the tables) the intracellular number of *Chlorella* cells was determined as described above. In some experiments ("chase"-experiments) the algae were incubated with the paramecia for only 30 sec ("pulse"), washed and kept in algae-free sterile medium for the period of time stated in Table 3. The numbers of SCVs formed were determined after fixation of the ciliates. Each value given in the tables is an average obtained from 40 to 60 ciliates in 2 to 4 experiments.

3. Results

When algae-free *P. bursaria* were brought together with cells of the exosymbiotically grown *Chlorella* sp. strain, most of the algae were taken up rapidly during the early phase of the experiment. About 80 cells were internalized during the first 5 min, about 160 cells were found within the host after a 30 min exposure, and around 300 cells after 24 hr (Table 1). Twenty-four hours after the end of the incubation period, only half of the infected ciliate population still contained algae, the other half had lost them by digestion. The infected paramecia contained, on an average, 2 algal cells only. The number of algae increased significantly when the internalized algae began to divide, 2 days after the start of the experiment (Table 2). There was

Table 1. Relationship between the time of incubation of algae-free *Paramecium bursaria* with cells of symbiotic *Chlorella* sp. and the success in establishing a permanent symbiosis between the two partners. Percentage of *P. bursaria* containing algae and mean number of algae per infected *P. bursaria* were scored 24 hr after the end of incubation.

Incubation periods of symbiotic *Chlorella* sp. with aposymbiotic *P. bursaria*	Number of ingested algae per *Paramecium* cell	Percentage of *P. bursaria* containing algae	Mean number of algae per infected *P. bursaria*
5 min	80	42	1.8
7 min	90	37	2.3
10 min	80	57	1.9
15 min	100	50	2.2
30 min	160	53	1.6
12 hr	300	–	–
24 hr	300	44	4.2
48 hr	300	49	14.5

Table 2. Percentage of *Paramecium bursaria* and numbers of algae found per ciliate 5 days after the incubation of algae-free ciliates with cells of symbiotic *Chlorella* sp. Data are percent of ciliates which contain a given range of algae cells.

Incubation period of symbiotic *Chlorella* sp. with aposymbiotic *Paramecium bursaria* (min)	Algal cells per *P. bursaria* 5 days after incubation				
	0	1–25	26 – 50	51–100	more than 100
5	55	12	5	8	20
7	45	15	17	11	12
15	52	12	12	5	19
30	54	9	8	16	13
40	66	3	8	6	17

Table 3. Relationship between the appearance of single-cell-vacuoles (SCV) within *Paramecium bursaria* and the time after the uptake of cells of symbiotic *Chlorella* sp. by the algae-free ciliates. Ciliates were incubated with algae for 30 sec, washed and fixed at different times after the end of incubation.

Time after the end of the incubation (min)	Percentage of SCV which contained algae	Percentage of ciliates with SCV	Localization of SCV within the ciliate cell
0	0	0	–
1.5	1.3	6	mainly at the posterior end
3	5.5	31	mainly at the
5	7.5	33	anterior end
7	9.3	48	and in the
15	14.3	76	middle part

no transfer of algal cells from infected to uninfected paramecia and the percentage of alga-free ciliates remained the same irrespective of the duration of ciliate-algae incubation. After 5 days, some ciliates contained only 25 algae, others possessed more than 200.

Besides the differences in intramembrane particle density (mentioned above), the most significant feature of the PV is the enclosure of just one single algal cell in contrast to the presence of several algal cells within a DV. Consequently, formation of a PV must start with the enclosure of a single alga in an individual vacuole (single-cell-vacuole, SCV). Therefore, we studied the number of SCVs and the sequence of their appearance after the uptake of algae by alga-free paramecia ("chase"-experiments). During the incubation with the algae, every *Paramecium* formed 1 to 10 vacuoles, each of which contained several algae. The average of the newly formed vacuoles per *Paramecium* was 4.6. As can be seen in Table 3, the number of SCVs per ciliate increased with time after the end of the incubation of the paramecia with the algae. The number of ciliates which contained SCVs also increased in the same manner. During the early phase of the experiments, vacuoles containing several algae and also SCVs were located mainly at the posterior end of the cell. Later on they were found predominately at the anterior end and in the middle part of the cell.

4. Discussion

In *P. caudatum* DVs undergo several successive steps during cyclosis (Allen, 1984) (Fig. 1). Vacuoles are formed at the cytopharynx from dis-

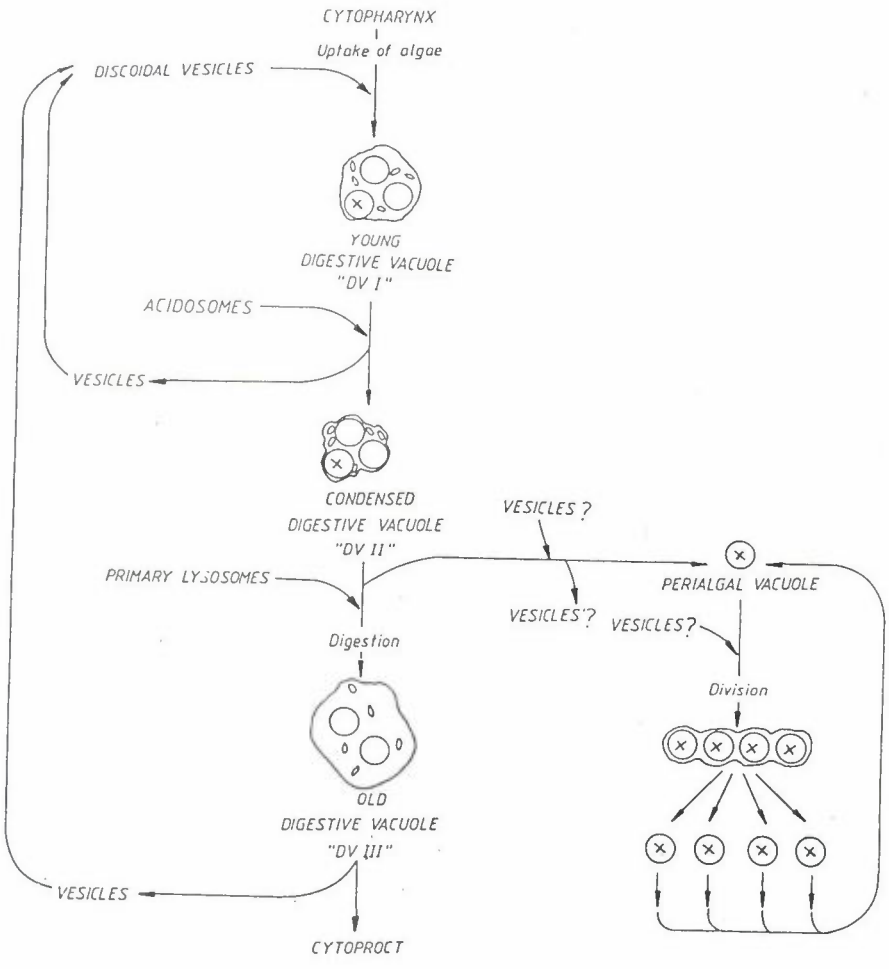

Figure 1. Schematic drawing illustrating the possible sequences of events which lead to the formation of perialgal and digestive vacuoles in *Paramecium bursaria*. Scheme adopted from Meier (1983). Results on the formation of digestive vacuoles in *Paramecium caudatum* (Allen, 1976; Allen, 1984) are included.

coidal vesicles. Fusion of these young vacuoles (DV I) with acidosomes and condensation leads to the DV II stage. Fusion with primary lysosomes, which transport acid phosphatase, starts the digestion process. From the cytoproct, membrane material is recycled to the cytopharynx (Allen and Fok, 1984; Fok et al., 1986). According to Meier et al. (1984), cyclosis of DVs in *P. bursaria* proceeds in a similar way; the first step during algal uptake is their enclosure

into vacuoles which contain several algae. However, as far as the establishment of PVs is concerned, the formation of single alga containing vacuoles has never been observed directly at the cytopharynx. A considerable number of SCVs appear 3 min after incubation started. Since fusion of DV I with acidosomes is reported to occur at about 30 sec after the formation of a DV at the cytopharynx (Allen and Fok, 1984), this process apparently takes place prior to SCV formation. Localization of the vacuoles in the anterior and middle part of the ciliate at that stage fits well with the localization of DV II observed by Allen and Fok (1984). Localization of vacuoles as well as the time of the appearance of SCVs (results obtained from serial sections of infected *P. bursaria*, Meier in preparation) indicate that SCVs are separated from DV II during the condensation phase. This presumably is the first step of PV formation and recognition. However, the final decision about the fate of the individual alga, even though it is enclosed in a SCV, does not occur at this stage, because not every SCV at this developmental stage is protected against fusion with primary lysosomes. The number of SCVs observed very shortly after the uptake of the algae by the ciliate exceeds that present in the paramecia 24 hr later. This decrease in SCV number hints to the existence of DV cyclosis also with SCVs. The decision about the fate of the algal cell thus corresponds with the prevention of SCV fusion with primary lysosomes. If such a fusion is not prevented, the SCVs turn into DVs. The prevention of SCV fusion with primary lysosomes might be achieved by the fusion of SCV with special non-lysosomic vesicles and/or the incorporation of otherwise transported specific membrane material into the SCV membrane. The amount of the latter might be limited in aposymbiotic *P. bursaria*, so that only 1 to 3 SCVs change into PVs, whereas the others return to the DV cyclosis system. It will be the aim of further studies to clarify the mode of transformation of SCV into PV and to investigate the possible induction of the synthesis of the specific PV membrane component by the endosymbiont in the regreening ciliates (see also Ayala and Weis, 1984; Weis, 1983). After the establishment of the endosymbionts in regreened *P. bursaria*, the amount of the protecting membrane material does not seem to be a limiting factor. The more or less simultaneous formation of many autospores accompanied by the enlargement and division of PVs requires the provision of large quantities of protection PV membrane material in *P. bursaria*.

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