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Endosymbionts in *Paramecium*

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Abstract

Paramecium species are extremely valuable organisms to enable experiments for the reestablishment of endosymbiosis. This is investigated in two different systems, the first with *Paramecium caudatum* and the endonuclear symbiotic bacterium *Holospora* species. Although most endosymbiotic bacteria cannot grow outside the host cell as a result of their reduced genome size, *Holospora* species can maintain their infectivity for a limited time. We found that an 89-kDa periplasmic protein has an important function for *Holospora*'s invasion into the target nucleus, and that *Holospora* alters the host gene expression; the host thereby acquires resistance against various stresses. The second system is the symbiosis between *P. bursaria* and symbiotic *Chlorella*. Alga-free *P. bursaria* and the algae retain the ability to grow without a partner. Consequently, endosymbiosis between the aposymbiotic host cells and the symbiotic algae can be reestablished easily by mixing them. We now found four checkpoints for the reestablishment of the endosymbiosis between *P. bursaria* and the algae. The findings in the two systems provide excellent opportunities for us to elucidate not only infection processes but also to assess the associations leading to eukaryotic cell evolution. This paper summarizes recent progresses on reestablishment of the primary and the secondary endosymbiosis in *Paramecium*.

Keywords: *Chlorella*; Endosymbionts; Endosymbiosis; *Holospora*; *Paramecium*

Introduction

The Gram-negative bacteria belonging to the *Holospora* species are endonuclear symbionts of ciliates of the *Paramecium* species complex (Fokin and Sabaneyeva 1997; Fokin and Görtz 2009; Gibson et al. 1986; Preer 1969; Ossipov, 1973; Ossipov et al. 1975, 1980; Skoblo and Lebedeva 1986). *Holospora* belongs to the α-proteobacteria (Amann et al. 1991; Lang et al. 2005). To date, nine *Holospora* species have been described (Fokin et al. 1996). All species show species-specificity and nucleus-specificity in their habitats. They cannot be grown outside the host cell with ordinary culture media because of their reduced genome size. They show two different forms in their life cycle: a reproductive short form (RF, 1.5–2 μm long) and an infectious long form (IF, 10–15 μm long) (Fokin et al. 1996; Fujishima et al. 1990b; Görtz 1980; Görtz et al. 1989; Gromov and Ossipov 1981). The bacterium exists as a short RF cell and divides by binary fission in the host nucleus when the host is growing. However, the RF halts the binary fission and differentiates into an IF cell through intermediate forms when the host cell starves or when its protein synthesis is inhibited (Fujishima et al. 1990a; Görtz 1983). During this differentiation, the bacterium forms a distinctive structure, one-half of which

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contains the cytoplasm; the other half is a periplasmic lumen with an electron-translucent tip (Dohra and Fujishima 1999; Dohra et al. 1994; Fujishima and Hoshide 1988; Görtz 1980; Görtz and Wiemann 1989; Görtz et al. 1989; Iwatani et al. 2005; Abamo et al. 2008). The IF cells engulfed into the host digestive vacuoles (DVs) escape with the electron-translucent tip ahead and penetrate the target nuclear envelope with this special tip when the IF cells are mixed with paramecia (Fujishima and Fujita 1985; Fujishima and Kawai 2004; Görtz and Wiemann 1989; Iwatani et al. 2005). For that reason, this tip is designated as an "invasion tip" (Iwatani et al. 2005). Under a phase-contrast microscope, the cytoplasmic region appears dark, but the periplasmic region appears as a refractile region (Dohra and Fujishima 1999; Görtz and Dieckmann 1980). In the macronucleus-specific *H. obtusa* of *P. caudatum*, the IF cells form two distinctive nucleoids during differentiation (Fujishima et al. 1990a). This bacterium also changes the buoyant density and protein composition (Fujishima et al. 1990a) and the surface morphology of the outer membrane (Fujishima et al. 1990b) during differentiation. When the host divides again, the IF cells of *H. obtusa* are collected in a connecting piece of the dividing nucleus. Then, they are freed from the dividing nucleus by wrapping with the nuclear membrane, and eventually expelled from the host cytoproct (Wiemann 1989). On the other hand, the outer membrane of the RF has a stronger affinity to bind the host chromatin than the IF cells, so that the RF cells remain in each daughter nucleus when the host cell divides (Ehrsam and Görtz 1999; Fokin et al. 1996; Görtz et al. 1992; Wiemann 1989). When the macronucleus is filled with many IFs, the host cells cannot grow and is killed by the bacteria. The IF cells that appeared outside the host cell by these two means can then infect new host cells through a DV. Because a *Paramecium* cell has a limited life span, holosporas must escape the host to infect younger cells. For that reason, a different nature of the outer membranes of these two forms is indispensable for *Holospora*'s survival.

The phenomenon of bacterial invasion into a target nucleus is designated as "infection". On the other hand, stable multiplication of the infected bacteria for more than two weeks is designated as "maintenance" (Fujishima and Fujita 1985). The infection is controlled by (1) engulfment of the IFs into the host DVs (Fujishima and Görtz 1983), (2) escape from the DV membrane before the host's lysosomal fusion happens in the host cytoplasm (Görtz and Dieckmann 1980; Iwatani et al. 2005), (3) migration to the target nucleus by means of the host actins (Fujishima 2009; Fujishima et al. 2007; Sabaneyeva et al. 2009), (4) recognition of a target nuclear envelope by a specific binding between *Holospora*'s outer membrane and their target nuclear envelope (Fujishima and Kawai 2004) and by a penetration of the host nuclear envelope by the invasion tip (Iwatani et al. 2005). On the other hand, the maintenance is controlled by the host genotypes (Fujishima and Mizobe 1988). Thus, the infection and the maintenance are independently controlled phenomena. The IF infects their target nucleus within 10 min after mixing (Fujishima and Görtz 1983). To date, the only organism having an ability to distinguish between the two kinds of host nuclei is *Holospora* species. Therefore, molecules responsible for this ability have received the attention of many researchers. Apparently, these bacteria "know" some differences of the nuclei originated from a common fertilization nucleus. After infection, the host cells change their gene expressions (Hori and Fujishima 2003; Hori et al. 2008; Nakamura et al. 2004), and acquire various stress resistances (Fujishima et al. 2005; Hori and Fujishima 2003; Hori et al. 2008; Smurov and Fokin 1998). In the first half of this paper, the molecular mechanisms underlying infection by *Holospora* of their target nuclei, and changes of the host cell that occur because of the infection are described.

In the completely different system of *P. bursaria*, the alga-free paramecia and the symbiotic algae still retain the ability to grow without a partner, and soon establish a mutual endosymbiosis after mixing them (see reviews, Kodama and Fujishima 2009c, 2010a,b).

Alga-free *P. bursaria* can be produced easily from alga-bearing cells by several methods (Hosoya et al. 1995; Jennings 1938; Karakashian 1963; Kodama and Fujishima 2008; Kodama et al. 2007, 2011; Pado 1965; Reisser 1976; Tanaka et al. 2002; Weis 1969, 1984; Wichterman 1948). Irrespective of mutual relationships between *P. bursaria* and symbiotic algae, the alga-free paramecia and the symbiotic algae still retain the ability to grow independently. Furthermore, endosymbiosis between the alga-free *P. bursaria* cells and the symbiotic algae isolated from the alga-bearing *P. bursaria* cells is easily reestablished by mixing them (Karakashian 1975; Siegel and Karakashian 1959). However, the mechanisms and timings used by the algae to escape from the host DVs and the differentiation into the perialgal vacuole (PV) membrane from the DV membrane wrapping the escaped alga could not been revealed for a long time. Recently, important cytological events needed for establishing endosymbiosis and their timings in the infection process were clarified by pulse-labeling with symbiotic *Chlorella* cells isolated from the alga-bearing *P. bursaria* for 1.5 min; then chasing for various times (Kodama and Fujishima 2005). *P. bursaria* is now becoming a model organism for studying the induction of secondary symbiosis, because the endosymbiosis can be induced synchronously (Kodama and Fujishima 2005). Moreover, all process of the endosymbiosis are observable under a light microscope. Furthermore, the nuclear genome of the symbiotic *Chlorella variabilis* was recently sequenced (Blanc et al. 2010). In the latter half of this paper, these four checkpoints for establishing a stable endosymbiosis between *P. bursaria* and the symbiotic *Chlorella* species are reviewed.

Holospora and *Chlorella* provide an excellent opportunity for us to elucidate not only for infection processes but also to assess the associations leading to eukaryotic cell evolution.