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REVIEW ARTICLE

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Intracellular bacteria in ciliates

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Abstract Ciliates are frequently colonized by other micro-organisms. The large size of ciliate cells offers habitats for hundreds to thousands of bacteria in different compartments, such as cytoplasm, nuclei and even perinuclear spaces. Size, phagocytic feeding habit and other features appear to be favorable pre-adaptations of ciliates for symbiosis with bacteria. Certain intracellular bacteria are permanent symbionts that are not infectious, whereas others are highly infectious. Both types show specific adaptations. With their wide spectrum of phylogenetic positions, intracellular bacteria in ciliates show relationships to different taxa of free-living bacteria and even archaea. Certain symbionts may be deleterious for their host ciliates, whereas others may provide a selective advantage under appropriate conditions or even be essential for the host cells. Depending on the nature of a symbiont, its prevalence in a host population may be low or high. Symbionts that express a killer toxin affecting non-infected ciliates achieve high infection rates in a host population, whereas certain infectious bacteria may only show a low prevalence.

Keywords Ciliates · Intracellular bacteria · Symbionts · Killer toxins

The ciliate cell

Ciliates are highly evolved heterotrophic protozoa [23]. As phagotrophic predators on micro-organisms, they bear the risk of bacterial infections: bacteria may resist digestion, escape from the phagosomes and colonize the cells. Infectious bacteria usually enter their host cells by phagocytosis, via the oral apparatus. An exception has been described by Soldo et al. [43], who found that the symbionts in *Parauronema acutum*, termed xenosomes, penetrate the cell membrane at sites of the cortex other than the oral apparatus. This is surprising, as the cortex of ciliates shows a strong, complex organization that may not easily be passed or perforated. Beneath the cell membrane, flat alveols tightly encircle the cytoplasm. Microtubules and other cytoskeletal structures form dense networks underneath the alveols, preserving the shape of the cell [1, 4].

Ciliates are large, unicellular organisms and, due to their size and cellular organization, they offer a variety of suitable habitats with more or less plenty of space for bacteria (Fig. 1). In the cytoplasm, bacteria may either prevail in symbiontophorous vesicles or be naked – not encircled by host membranes. Symbionts may live in the large, somatic macronuclei and in the much smaller, generative micronuclei. Bacteria have even been found in the perinuclear space and in the endoplasmic reticulum. The ciliate cell thus may be regarded as a microcosm that may harbor different symbionts at the same time.

Ciliates multiply by binary division and sexually propagate by conjugation. The latter includes meiosis of the micronucleus, resorption of the old macronucleus and formation of a new macronucleus from the synkaryon that will also give rise to the new micronucleus. Whereas cytoplasmic symbionts may be simply distributed to daughter cells during binary division, intranuclear symbionts may even make use of the nuclear division machinery. Both micronuclear mitosis and macronuclear division are closed divisions: the nuclear envelopes are maintained, keeping nucleoplasm and cytoplasm separated throughout the cell cycle. Endonuclear symbionts are therefore caught in the nuclei, unless they have developed means of crossing the nuclear envelopes. During conjugation of the host cell, resorption of the old macronucleus may be deleterious for intranuclear symbionts that have not adapted to this process; and the bacteria are digested in the process of resorption. New macronuclei being developed after conjugation are free of bacteria before being infected anew.

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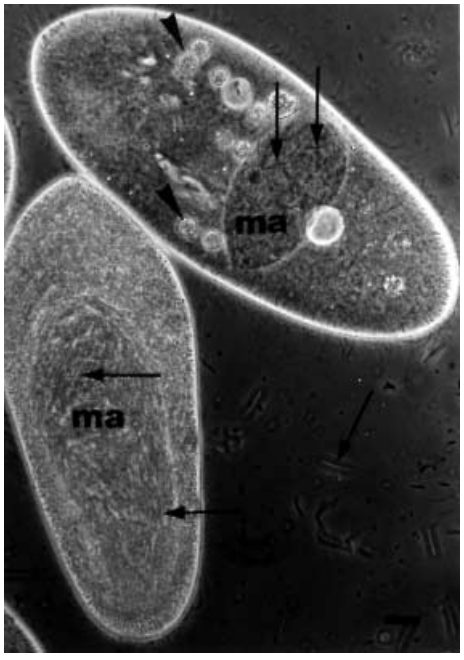

Fig. 1 *Paramecium caudatum* with *Holospora obtusa* in the macronucleus. The macronucleus of the left cell is heavily infected; and, apparently, this cell is affected and no longer feeds, as no food vacuoles are visible in the cell. *Arrowheads* Food vacuoles, *arrows Holospora* bacteria, some infectious forms in the medium, *ma* macronucleus. Phase contrast, microflash. *Bar* 10 μm

In spite of the problems arising for bacteria colonizing nuclei, endonuclear symbionts are frequently found in ciliates. This seems to be due to three reasons: (1) in the nuclei, symbionts might be provided with the most complete supply of metabolites, (2) in cell divisions, the distribution of symbionts to the daughter cells might be better ensured in the nuclei and (3) in the nuclei, cellular defense mechanisms against bacteria appear to be unlikely. Symbionts in the nuclei are naked – not encircled by host membranes – and attacks by lytic enzymes would be deleterious to chromatin, too.

Adaptations and types of symbionts

Traditionally, intracellular bacteria in ciliates are called symbionts, based on the original definition of symbiosis by DeBary from 1897 as the living together of heterospecific partners. Although symbionts in ciliates are often cited as good examples for a possible development of cell organelles from symbiotic bacteria, little is known about metabolic interactions of intracellular bacteria and their host ciliates. Symbionts are susceptible to antibiotics and other treatments may also kill them. Curing the ciliates of their infections is not detrimental to the host cells in most cases; and the significance of most symbionts for their hosts is unclear.

An exception to some degree are the killer symbionts. Intracellular bacteria in some ciliates, namely *Paramecium*, provide their hosts with the ability to kill individuals of the same or closely related species that do not bear such symbionts. It is known from *Caedibacter taeniospiralis*, originally called kappa-particles [44], that the toxic principle is associated with the bacteria being released from their host cells into the surrounding medium (for reviews, see [25, 36]). Killer symbionts provide their hosts with resistance against the toxin. A resistance against killer toxins is also provided by bacterial symbionts that do not produce toxins [36, 40]. Neither the nature of the toxins nor the mechanism of resistance have been identified.

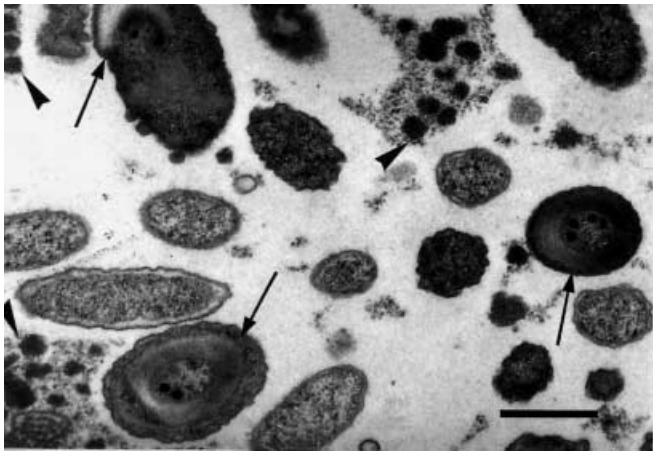

Fig. 2 *Caedibacter caryophilus* in the macronucleus of *P. caudatum*. *Arrowheads* Host chromatin, *arrows* R bodies with phage-capsids. Electron micrograph. *Bar* 0.5 μm

At least in some killer symbionts, the production of toxin is related to the presence of plasmids or phages. In *C. taeniospiralis* and other species of the genus, refractile bodies called R bodies are produced (Fig. 2), whose proteins are encoded by plasmids of phage genomes and appear to be involved in the toxicity [35, 38]. These proteinaceous R bodies are ribbons coiled inside the symbiont to form a hollow cylindrical structure of up to 0.8 μm in diameter [35, 38]. R bodies may unroll and destroy phagosomal membranes when ingested and, by this or subsequent actions, they kill sensitive cells [36].

Three genes that are independent transcriptional units on a plasmid in *C. taeniospiralis* have been characterized and sequenced by Heruth et al. [27]. The genes (*rebA*, *rebB*, *rebC*) are involved in the synthesis and assembly of R bodies. Two polymerization events have been found to be involved in R body assembly: one event requires RebB and RebC; and the other requires all three proteins. It appears that RebC is involved in post-translational modifications of RebA and RebB, both of which show peptide species with different molecular weights. The gene coding for a fourth protein has not been sequenced yet and its function remains unknown [27].

Although many killer symbionts with and without plasmids or phage-capsids do not produce R bodies, they express toxic activities ([36], unpublished observations). Also, killer symbionts are not limited to

Paramecium. More detailed investigations were also done in the marine ciliate *Parauronema acutum* [41]. Killer bacteria in this ciliate are infectious, obligate symbionts. In this case, the killer effect seems to be mediated by a protein also encoded by bacterial extrachromosomal DNA [41]. Both non-killer and killer symbionts of *Parauronema* possess extrachromosomal DNA. Whereas two different plasmids are present in the non-killer bacteria, four plasmids are found in the killer bacteria. All of these contain a homologous region of about 17 kbp [41]. When a symbiont-free ciliate that had harbored killer symbionts was infected with non-killer symbionts, the ciliate switched to become an active killer again. This provides evidence that plasmid DNA from the killer symbiont had remained in the ciliate and entered the non-killer bacterium, transforming it into a killer symbiont again. It is one of the few examples in ciliates in which symbiont DNA may be transferred to the host cell and be maintained.

Like rickettsias, which are pathogens of humans and animals, most intracellular bacteria in protozoa cannot grow extracellularly on artificial media, but appear to be obligate endocytobionts. Some of them have been shown to depend upon certain host genes. Host cells that are heterozygous (e.g. Kk for the K-gene) contain half the number of symbionts as homozygous (KK) cells [14, 36]. The functions of such genes are not known.

Advanced adaptations typical for intracellular bacteria are found in symbionts of the freshwater ciliate *Euplotes* [25]. In these ciliates, bacteria of the genus *Polynucleobacter* are spread throughout the cytoplasm. The type species of these symbionts, *P. necessarius*, has been found in *E. aediculatus* [24]. Ciliates cured of their symbionts die and reinfections with *P. necessarius* or certain related symbionts may save the ciliates [15]. *P. necessarius* and related symbionts are obligate endocytobionts and cannot grow outside their hosts. Obviously, they need a complex substrate with metabolites which is provided by their hosts. Thus, *Polynucleobacter* and its relatives are intimately adapted to their hosts and vice versa. Unlike rickettsias, these symbionts are neither infectious nor pathogenic, but rather function like an organelle. However, it is not known what metabolites are provided by the symbionts.

The fact that bacterial symbionts have been found in all fresh-water *Euplotes* of a certain taxonomic group suggests that the species of this group suffer from a metabolic deficiency that arose in a common ancestor [26]. *P. necessarius* and *P. necessarius*-like symbionts are considered to be progeny of an early symbiont that compensated for this metabolic deficiency. Whereas the origin of this symbiosis appears more recent than the origin of mitochondria and chloroplasts, it may be older than many other kinds of symbioses between bacteria and ciliates, because similar symbionts are found in many *Euplotes* species from different habitats.

Differing from most free-living bacteria, *P. necessarius* in *Euplotes*, xenosomes in *P. acutum* and some other bacterial symbionts and pathogens possess small, multicopy genomes capable of encoding relatively few proteins [39, 41]. The symbionts seem to have lost many genes of their ancestors during the coevolution with the ciliates. Being nourished with metabolites of the host cell, the symbionts should only have to retain those genes essential for their own survival in intracellular environments and certain genes needed for the synthesis of metabolites that are essential for the host cells (for reviews, see [36, 41]). This process of coadaptation may not only have resulted in a reduction of genome size of the symbiont but also in a transfer of bacterial genes to the host genome. Nevertheless, direct evidence of the transfer of a nuclear gene of bacterial origin has not been found for ciliates.

Infectious bacteria

Certain intracellular bacteria of protozoa are highly infectious. As a rule, infectious bacteria in ciliates are neither host-specific nor compartment-specific: they invade either the cytoplasm or the micronucleus or macronucleus. Bacteria of the genus *Holospora* invade the nuclei of various species of *Paramecium* [22, 25]. Related bacteria infect other ciliates (unpublished observations).

Holospora shows a developmental cycle; and, at various stages in this cycle, the bacteria have different morphologies (Figs. 3, 4). After divisions of the host cell, specialized infectious forms are released into the medium, whereas morphologically different reproductive forms are maintained within the host nuclei. Reproductive forms multiply in the nuclei and, in each nucleus, some reproductive forms may develop into infectious forms again.

A new infection originates from the ingestion of an infectious form by a paramecium. The bacterium leaves the phagosome, is transported through the cytoplasm and is taken into the host nucleus by fusion of the two membranes of the transport vesicle with those of the

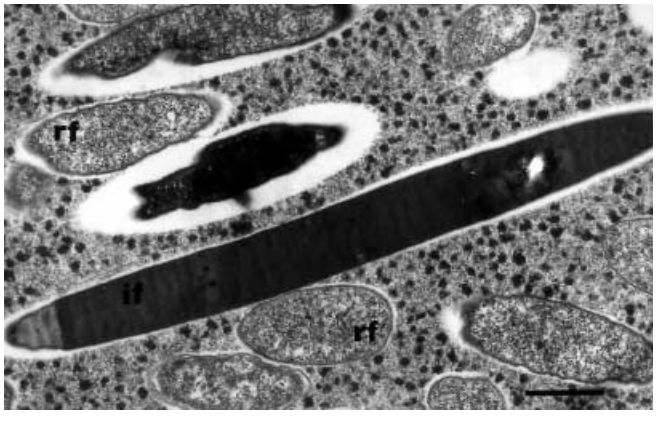

Fig. 3 Macronucleus of *P. caudatum* infected with *H. obtusa*. *if* Infectious form, *rf* Reproductive form. Electron micrograph. *Bar* 1 μm

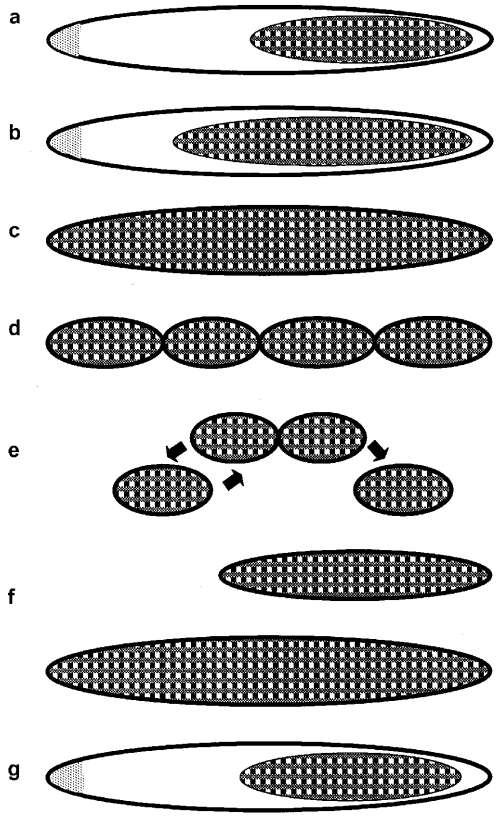

Fig. 4a–g Developmental cycle of *H. obtusa*. Getting into the phagosome of *Paramecium* during invasion, the cytoplasm of the infectious form extends into the periplasmic space (**a**, **b**) and, in the host nucleus, the cytoplasm fills the whole cell (**c**). The bacterium then divides into small cells, establishing the reproductive form (**d**); and the reproductive multiplies by binary division (**e**). Later, some of the reproductive forms may grow longer (**f**) and develop into the infectious forms by depositing proteins into the periplasm; and the cytoplasm is condensed (**g**)

nuclear envelope. Transport to the nucleus and within the nucleus during its division appears to be mediated by the cytoskeleton of the host cell [19, 33].

The expression of a developmental cycle itself and, perhaps even more, the organization of the infectious form are highly evolved adaptations. The infectious form of *Holospora* has a voluminous periplasm that contains several stage-specific proteins, some of which appear to be released during the infection process [5, 16, 19, 20, 48]. Some proteins that are immunolocalized in the periplasm of the infectious form become localized on the surface of the bacteria after their ingestion into the phagosome or are found to be associated with the phagosomal membrane. This is taken as evidence that such proteins are used for communication with membranes and other structures of the host cells. Released periplasmic proteins could also protect the bacteria against lytic enzymes or inactivate such enzymes. Sugar-binding activities and glyco-residues, which might be part of a communication system of host and symbiont, have been found on the bacterial surface.

The gene of a small periplasmic protein of 5.4 kDa has been sequenced [6]. Northern blot hybridization showed that the gene is highly expressed in the intermediate form, a transitional stage in the development from the reproductive into the infectious form of the bacterium. Amino acid sequence similarities with other peptides have not been found [6]. It is suggested that the protein may function in the recognition process during the early phase of infection. Dohra et al. [7] have also identified a GroEL-like protein in *H. obtusa*. The gene is selectively expressed in the reproductive form. Whereas the functions of the 5.4-kDa protein and other proteins/genes (unpublished results) of *H. obtusa* have not yet been determined, certain proteins/genes have been found to be part of the secretory machinery of the bacteria (unpublished results). An effective secretory pathway seems to be of special significance for infectious bacteria being important to control the release of invasion proteins and pathogenicity factors [37].

Symbiotic methanogens in anaerobic ciliates

Ciliates of anaerobic environments, such as the rumen or the sapropel are often associated with intracellular or epibiotic methanogens [8, 9, 10, 47]. Also, ciliates and other protozoa found in the gut of arthropods and other invertebrates may be associated with methanogenic symbionts [21]. Anaerobic protists generate their energy by converting carbohydrates to lactate, acetate and butyrate. Reducing equivalents are removed in the form of H_2, which is produced by proton reduction involving the enzyme hydrogenase [32] that is found in the hydrogenosomes of anaerobic protozoa [49]. Hydrogenosomes are microbody-like organelles characterized by their pyruvate synthase and hydrogenase activity, indicating the conversion of pyruvate into acetyl-CoA plus H_2 and CO_2. For the optimal function of the enzyme hydrogenase, the concentration of H_2 must be kept low (< 1 kPa). This is ensured by the methanogenic bacteria consuming H_2 and converting it with CO_2 to methane. As a morphological prerequisite for H_2 transfer, endosymbiotic methanogens may be closely associated with the hydrogenosomes of their host cells. The methanogenic symbionts of *Plagiopyla frontata* even divide synchronously with their host cell [8]. The H_2 transfer between anaerobic ciliates and methanogenic bacteria therefore appears truly mutualistic.

Methane is one of the greenhouse gases, much of which is released into the atmosphere by intracellular microorganisms. Hackstein and Stumm [21] calculated a possible production of 2×10^8 t of methane by arthropods and a production of $8–10\times10^8$t by ruminants each year. Most of the methane in the arthropod gut is produced by endocytobiotic methanogens in ciliates [21]. Finlay et al. [10] calculated that up to 37% of the methane production in a sheep may come from methanogenic ciliates.

Phylogenetic diversity of intracellular bacteria

With a few exceptions, bacterial symbionts in ciliates were given binomial names only in the 1970s [36], when the last doubts were overcome that the symbionts were indeed bacteria. At that time, chiefly biological and morphological features were used to describe the species, as metabolic and molecular data were still scarce. In the 1990s, analysis of ribosomal genes brought great progress in our knowledge about the phylogeny of intracellular bacteria in protozoa (e.g. [2, 12, 28, 45, 46]). We should remember that the first intracellular bacteria in ciliates had been named 100 years ago. It was Hafkine who introduced the genus *Holospora* and the three species *H. obtusa*, *H. elegans* and *H. undulata*, all of them infecting the nuclei of *P. caudatum* [22]. Now, sequencing of ribosomal and other genes will help to clarify unresolved or misinterpreted phylogenetic positions of many bacterial symbionts.

Among ciliates, far most intracellular bacteria have been observed in the genus *Paramecium* [25, 36]. However, recent searches for bacteria in ciliates other than *Paramecium* show that the great diversity of intracellular bacteria in this host may not be an exception. Many bacterial symbionts have been found in *Euplotes* [25], though most of them are poorly investigated; and only in a few isolates of *Stentor* and *Spirostomum* have several unknown bacteria been observed (unpublished results). Having in mind also the diversity of bacteria found in amebas as another group of protozoa that is now being investigated for bacterial symbionts [28], it is suggested that most protozoa should have a variety of specific intracellular bacteria. The conclusion seems justified that the number of intracellular bacteria in protozoa may be much higher than the number of protozoan species.

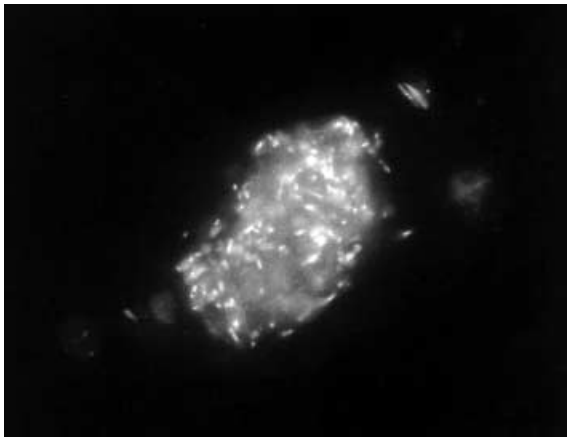

Fig. 5 *H. obtusa* in the macronucleus of *P. caudatum*. In situ hybridization using a *Holospora*-specific probe labeled with Cy3. Fluorescence micrograph. *Bar* 5 μm

The detection of new microorganisms from nature has been accelerated by PCR amplification of ribosomal sequences. Concerning intracellular bacteria, however, this method suffers a high level of confusion, due to contaminants that are associated with eukaryotic cells but are not really intracellular. This is especially true in the search for intracellular bacteria in protozoa. Protozoa feed on free-living bacteria and some of the bacteria ingested are slowly digested or not digested at all. It is therefore necessary to prove that the ribosomal sequences amplified and sequenced come from the intracellular bacteria and not from prey organisms or any contaminants. The PCR detection of new intracellular microorganisms in protists is therefore only reliable with a subsequent verification of the results. This verification may be done by in situ hybridization, using labeled oligonucleotide probes that have been designed according to PCR-amplified sequences. Amann et al. [2, 11, 45] have shown that, by in situ-hybridization, even bacteria inside their host cells can be detected and, according to the sequence of the probe, may be identified as belonging to a certain taxon (Fig. 5).

The introduction of in situ hybridization using fluorochromated, specific oligonucleotide probes to detect intracellular bacteria was certainly a big step forward in the investigation of intracellular micro-organisms. The few examples we have up to the present (Table 1) already show that intracellular environments in protozoa may offer appropriate conditions for most different taxa of bacteria. The observation that various intracellular symbionts in protozoa are related to pathogens may point out the risk that new pathogens could emerge from intense contact with protozoa to which the human immune system is not accustomed [17]. Sequence analysis of amplified genes of intracellular bacteria may also provide further insights into the genome structure of these organisms. An analysis of the 16S rDNA of *Caedibacter caryophilus*, a killer symbiont dwelling in

Table 1 Phylogenetic positions of some intracellular bacteria in ciliates and other protozoa

Host	Symbiont	Phylogenetic position	Ref.
Paramecium caudatum	*Holospora obtusa*	α-Proteobacteria	[2]
P. caudatum	*H. elegans*	α-Proteobacteria	[2]
P. caudatum	*Caedibacter caryophilus*	α-Proteobacteria	[46]
Euplotes aediculatus	*Polynucleobacter necessarius*	β-Proteobacteria	[47]
Acanthamoeba polyphaga	*Candidatus C. acanthamoebae*	α-Proteobacteria	[28]
Acanthamoeba sp.	*Candidatus Paracaedibacter symbiosus*	α-Proteobacteria	[28]
Acanthamoeba sp.	*Candidatus Parachlamydia acanthamoebae*	Chlamydiaceae	[3]
Metopus contortus	*Methanoplanus endosymbiosus*	Methanobacteria	[47]

the macronucleus of *Paramecium caudatum*, revealed an unusual insertion of 194 bp that was not present in mature 16S rRNA [45]. It was shown that *C. caryophilus* contained fragmented 16S rRNA.

The phylogenetic diversity of intracellular bacteria in ciliates and other protozoa emphasizes again that unicellular organisms, especially if they are phagotrophic, bear great risks of being infected by other microorganisms. Adaptations subsequent to an infection may occur easily and quickly. The most amazing example of rapid adaptation following a new infection is that described by Jeon [29]. He observed an infection of *Amoeba proteus* by bacteria that later became essential for the host cells: the ameba became dependent upon the new symbionts.

Ecological significance of intracellular bacteria

Intracellular bacterial infections in protozoa give many examples for the significance of symbiosis in evolution. The cooperation of two or more genomes, possibly resulting in a gene transfer from symbiont/organelle to host nucleus may be of advantage in competition with aposymbionts under many environmental conditions [31]. Even though gene transfer from symbiont to host has not been detected in ciliates, intracellular bacteria may influence the metabolism, behavior and fitness of host cells and host populations and will be of significance for food webs and ecosystems. However, up to the present, we have little information about the implications of these symbioses. The killer trait has been taken as a typical example of mutual symbiosis. It is argued that ciliates with killer symbionts have an advantage over individuals of the same or closely related species competing for the same resources. Landis has demonstrated that at least in the case of *C. taeniospiralis* in *P. tetraurelia* and *C. varicaedens* in *P. biaurelia*, natural selection is the mechanism which maintains the killer trait, whereas the hosts have evolved mechanisms to ensure the maintenance of killer symbionts in their populations (for a review, see [30]). Our own observations of a natural population of *P. caudatum* show infection rates with the endonuclear bacterium *C. caryophilus* of 100% over many years (unpublished). This corresponds with observations in laboratory cultures: symbiont-free cells are killed and cultures always remain infected to 100%.

In spite of the high infection rates of *P. caudatum* with endonuclear killer symbionts, the nature of the symbiosis seems to be different from that of cytoplasmic killer symbionts in *P. aurelia* species. A comparison of the growth rate of symbiont-free *P. caudatum* with that of paramecia bearing *C. caryophilus* reveals that symbiont-free cells grow faster. Under unfavorable conditions, such as starvation, paramecia are even killed by *C. caryophilus*, because the bacteria keep on multiplying, even when the host cell starves. Infected macronuclei are finally overgrown by the bacteria. These observations indicate that bearing *C. caryophilus* is costly for *P. caudatum* and may be of questionable advantage.

Clear evidence of the selective advantage of intracellular bacteria for their host cells was obtained for *Polynucleobacter necessarius* in *E. aediculatus* [24, 25]. In contrast to *Lyticum flagellatum*, a killer symbiont of *Paramecium octaurelia*, where Soldo et al. [42] found evidence that the bacteria may provide their host with folic acid, we have no knowledge about the metabolic function of *Polynucleobacter necessarius* for *Euplotes*. Yet, it is obvious that the symbiont is essential for its host. Although sequence data have been obtained only for *P. necessarius*, it appears from cytological investigations that the symbionts in *Euplotes* related to *E. aediculatus* bear *P. necessarius*-related bacteria [26] and apparently, all of them depend upon their symbionts. This even holds true for *E. daidaleos*, a green ciliate that harbors *Chlorella*-symbionts in addition to the bacteria [26].

In contrast to the killer symbionts that simply kill uninfected ciliates, in the case of *P. necessarius* and related symbionts, it is their essential role for *Euplotes* that ensures the infection of the ciliate populations. In both cases, host populations are completely infected. This may be different with infectious bacteria. Infectious symbionts may be maintained even in the face of selective pressure. Accordingly, *Holospora*, which may be regarded as a true parasite, presents stable infection rates in natural populations of *Paramecium*.

Infectious forms of *Holospora* are regularly released by the host cells into the surrounding medium and may infect new host cells. If the macronucleus (this is the somatic, transcriptionally active nucleus of ciliates) is infected, this may affect the growth rate and, under unfavorable conditions, host cells may even die. If conditions are favorable, host cells appear not to be damaged. Some natural populations were found to be infected over years, with infection rates close to 100%. This is different for micronuclear-specific infections. The micronucleus being the generative nucleus of ciliates, it maintains the complete genome for sexual propagation over many divisions. In an infection of the micronucleus with *H. elegans*, conjugation (sexual propagation) is no longer successful [18]. Infected host cells form pairs and infected micronuclei divide meiotically, but exconjugant cells are not viable. Apparently, no functional macronuclei can be established. The inhibition of successful conjugation renders the host genetically dead and therefore may be reminiscent of the parasitic castration of metazoa that is caused by certain parasites. Consequently, infection rates may not be high in cases of micronuclear infections. They are usually lower than 10% in natural populations.

Holospora does not necessarily kill its host cell. Rather, bacteria and host appear to be well adapted in many host strains. In starving paramecia or after inhibition of host protein synthesis, most bacteria differentiate into the infectious form [13], which is a resting stage and does not multiply. Although populations of

Paramecium are frequently infected by *Holospora*, infection rates are often low, especially in the case of micronuclear infections. Also, paramecia seem to have mechanisms to cure themselves of endonuclear symbionts. It was discovered that *Holospora* may synchronously and completely lyse in the host nuclei of certain strains of *Paramecium* after an infection [11, 34]. Bacterial lysis in such cases may be due to unknown defense-mechanisms against intracellular infections evolved in ciliates. After lysis of the bacteria, host cells are cured and remain viable.

Natural populations of ciliates may be infected by different bacteria; and even two or more different symbionts can colonize individual cells. Whereas some intracellular bacteria appear to prevent further infections of their host cell, other symbionts even seem to favor each other or at least do not exclude growth of other bacteria [36]. Observations of double infections, where infectious and non-infectious bacteria coexist, suggest that infectious bacteria may guide non-infectious bacteria into the nuclei of potential host cells. Experimental evidence that infectious bacteria may serve as vectors for non-infectious bacteria was recently obtained by Fokin and colleagues (unpublished observations). Free-living bacteria such as *Enterobacter aerogenes* and weakly infectious symbionts (*Nonospora macronucleata*) were co-transported together with the highly infectious *H. obtusa* into the macronucleus of *P.caudatum*.

Conclusions

Intracellular bacteria found in ciliates and other protozoa show a great diversity. Now that appropriate methods for their identification and phylogenetic classification are available, there is no question that the number of intracellular bacteria in protozoa will increase considerably in the near future. We may expect to find new mechanisms of bacterial infection, intercellular communication between host and symbionts and new mechanisms of cellular resistance against infectious microorganisms. Intracellular bacteria may influence the metabolism, behavior and fitness of host cells and host populations; and therefore they are of significance for food webs and ecosystems. The investigation of intracellular bacteria in ciliates and other protozoa will therefore also improve our understanding of the function of ecosystems.

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