Protoplasma (2005) 226: 147–153
DOI 10.1007/s00709-005-0121-7

PROTOPLASMA
Printed in Austria

Nocodazole inhibits macronuclear infection with *Holospora obtusa* in *Paramecium caudatum*

E. V. Sabaneyeva[1,*], **S. I. Fokin**[2], **E. V. Gavrilova**[1], and **E. S. Kornilova**[3]

[1] Department of Cytology and Histology, Saint Petersburg State University, Saint Petersburg
[2] Biological Research Institute, Saint Petersburg State University, Saint Petersburg
[3] Institute of Cytology, Russian Academy of Sciences, Saint Petersburg

Received January 20, 2005; accepted April 29, 2005; published online December 12, 2005

Summary. *Holospora obtusa* is a Gram-negative bacterium inhabiting the macronucleus of the ciliate *Paramecium caudatum*. Experimental infection with *H. obtusa* was carried out under nocodazole treatment. Nocodazole has been shown to cause disassembly of the cytoplasmic microtubules radiating from the cytopharynx and postoral fibers in *P. caudatum*. Treatment with this drug did not prevent the ingestion of both prey bacteria and *H. obtusa*, but it reduced the phagosome number and affected cyclosis. In situ hybridization revealed infectious forms of this endobiont very close to the macronucleus, but never inside it. These results indicate that disassembly of microtubules does not impair transportation of the infectious forms of *H. obtusa* in the cytoplasm, but that it completely blocks the invasion of the nucleus by the bacteria.

Keywords: Intracellular bacterium; Infection; Microtubule; Nocodazole; Transport; *Paramecium caudatum*; *Holospora obtusa*; Fluorescence in situ hybridization.

Abbreviations: Ma macronucleus; DV-I digestive vacuole I.

Introduction

Ciliates are known to be the hosts for numerous intracellular symbiotic organisms traditionally referred to as endobionts (Ossipov 1981; Görtz 1986, 1988; Fokin 1993, 2004). Endobionts can occupy various cell compartments, including the cell nuclei. One of the best studied endobiotic model systems is that of *Paramecium caudatum* and *Holospora obtusa* (Ossipov and Podlipaev 1977, Fujishima and Fujita 1985, Fujishima and Mizobe 1988, Görtz and Wiemann 1989, Rautian et al. 1990, Fokin et al. 2003, Fokin 2004). *Holospora obtusa* is a Gram-negative bacterium belonging to the α-proteobacteria and is an obligatory endobiont of the *P. caudatum* macronucleus (Ma). The life cycle of *H. obtusa* comprises an infectious (10–20 μm long) and a vegetative (2–3 μm) form, or stage. Six steps can be distinguished in the infection process (Fokin et al. 2003). The infectious form is ingested by a ciliate during the course of phagocytosis and enters a newly formed phagosome or, according to the nomenclature proposed by Fok and Allen (1988), digestive vacuole I (DV-I). Shortly after entering DV-I, the bacterium leaves the phagosome, obviously in the course of transformation of DV-I into DV-II as both processes are initiated by the slight acidification caused by the fusion of DV-I with acidosomes (Fok and Allen 1988, Fujishima 1996, Allen and Fok 2000, Kawai and Fujishima 2000). The bacterium evaginates the phagosome membrane, thereby forming the bacterium-bearing vesicle. The activated infectious form then starts its dangerous voyage through the host cell cytoplasm surrounded by a membrane originating from the phagosome membrane. It normally takes the bacterium about 30 min to reach the final destination compartment, the Ma. The activated infectious form is sluiced into the Ma (Ossipov and Podlipaev 1977, Görtz and Wiemann 1989, Kawai and Fujishima 2000), where it becomes fragmented and thus transformed into vegetative forms.

Although the *P. caudatum–H. obtusa* model has been extensively studied in many aspects, the process of bacterial transportation within the host cell cytoplasm still remains unclear. The host cell cytoskeleton has been suggested to be involved in the transportation of the bacterium-bearing vesicle through the paramecium cytoplasm (Görtz 1996). A tail trail-

* Correspondence and reprints: Department of Cytology and Histology, Faculty of Biology and Soil Sciences, Saint Petersburg State University, Universitetskaya naberezhnaya 7/9, 199034 Saint Petersburg, Russia.
E-mail: saban@mail333.com

ing after this vesicle, which strongly resembles that of listeria, can be observed in some electron micrographs (Görtz and Wiemann 1989). This latter observation led the authors to suggest the possible participation of actin microfilaments in bacterial transportation. At the same time, a number of other bacteria (*Citrobacter freundii*, *Campylobacter jejuni*, *Haemophilus influenzae*, *Klebsiella pneumoniae*) were shown to require microtubules for successful invasion (Oelschlaeger et al. 1993, Oelschlaeger and Tall 1997). The present study was undertaken to test whether microtubules play any role in the infection of the *P. caudatum* Ma by *H. obtusa* bacteria. Nocodazole, which is known to cause microtubule depolymerization, was used for this purpose. The results obtained here provide evidence for the participation of the host cell microtubular cytoskeleton in bacterial invasion of the Ma.

Material and methods

Cell origin and cultivation

The following strains of *Paramecium caudatum* were used in the present study. The endobiont-free strain PG-1 was isolated from nature by S. I. Fokin in Gatchina (St. Petersburg district, Russia). The endobiont-free strain RK-4 was isolated at the White Sea Biological Station Kartesh by S. I. Fokin. The strain RWK98-2H.o, bearing *Holospora obtusa* in the Ma, was isolated from Lake Krivoye in the vicinity of the aforementioned station by S. I. Fokin. The cells were maintained in a lettuce medium inoculated with *Enterobacter aerogenes* at room temperature.

Nocodazole treatment

Nocodazole was prepared as a stock solution (1 mg/ml) in dimethyl sulfoxide and added directly to the culture medium to obtain a final concentration of 10 μg/ml. To avoid nocodazole dilution through the addition of the homogenate in experimental infection, the drug was administered twice in equal portions. The first was added to the cell culture 15 or 30 min prior to experimental infection. The second was added simultaneously with the homogenate, maintaining the final nocodazole concentration at 10 μg/ml. Incubation with nocodazole was performed at room temperature and lasted from 40 min to 3 h. Control cells were treated either with dimethyl sulfoxide or with distilled water.

Immunocytochemistry

To check the extent of microtubular disassembly under nocodazole treatment in *P. caudatum*, an immunocytochemical study using monoclonal antibodies against α-tubulin DM 1A (Sigma) was performed. For antibody labeling, *P. caudatum* cells were treated according to the procedure described by Cohen and Beisson (1988) with slight modifications. Cells were collected in a small drop of medium and permeabilized in PHEM buffer (60 mM piperazine-N,N′-bis(2-ethanesulfonic acid), 25 mM HEPES, 10 mM EGTA, 2 mM $MgCl_2$) supplemented with 0.5% Triton X-100 for 1–3 min and then immediately transferred into 4% paraformaldehyde in phosphate-buffered saline (PBS) or PHEM. After 1 h of fixation, the cells were transferred to polylysine-coated slides and washed in PBS. The slides were incubated in PBS or Tris-buffered saline (TBS) containing 3% bovine serum albumin (BSA) for 30 min and then for 1 h in the presence of monoclonal antibodies against α-tubulin diluted 1 : 1000 in PBS–BSA or TBS–BSA. The cells were then washed in PBS, incubated in PBS or TBS containing 3% BSA for 30 min and treated with goat anti-mouse antibodies conjugated with Cy3 diluted 1 : 200 and mounted in an antifade solution containing 4′,6-diamidino-2-phenylindole to visualize the nuclei.

Slides were analyzed with a DMRXA fluorescent microscope (Leica) equipped with a charge-coupled-device camera and QFISH software (Leica Cambridge Ltd.) at the Scientific Research Center "Chromas", Biological Research Institute of St. Petersburg State University.

Phagosome counts

To test phagocytosis efficiency in nocodazole-treated cells, the homogenate was substituted in a series of experiments with fresh lettuce medium inoculated with prey bacteria containing Kongo red (4 μg/ml). Phagosomes were counted in the immobilized living cells. Paramecia were immobilized in a compression device for living cell observation (Skovorodkin 1990). Live cells were examined by phase-contrast or Nomarski differential interference contrast microscopy with a Zeiss Axioskop microscope.

Experimental infection

The homogenate was prepared from stably infected cells according to Preer (1969). Endobiont-free cells were infected by mixing equal volumes of cell culture and homogenate in a 3 ml well. To check the course of the infection, living cells were examined by Nomarski differential interference contrast microscopy with a Zeiss Axioskop microscope 30 min, 1 h, and 2 h after adding the homogenate.

In situ hybridization

Cells were fixed with 4% formaldehyde (w/v, freshly prepared from paraformaldehyde) in PBS, pH 7.2, for 2 h and then washed with PBS. Cells were incubated with oligonucleotide probes in hybridization buffer (Fokin et al. 1996). A eubacteria-specific probe, 5′-GCTGCCTCCCGTAG GAGT-3′, labeled with fluorescein isothiocyanate and a probe specific to the α-subgroup of proteobacteria, 5′-GCGTTCGCTCTGAGCCAG-3′, labeled with tetramethylrhodamine isothiocyanate (Amann et al. 1990, 1991) were used. Specimens were investigated with a Zeiss LSM 410 confocal laser scanning microscope equipped with a Plan-Neofluar 100× oil immersion objective. For the detection of fluorescein and tetramethylrhodamine isothiocyanate, an argon-ion laser (488 nm wavelength) and a helium-neon laser (543 nm wavelength) with appropriate emission filters (BP, 510–525 nm and 575–640 nm wavelength, respectively) were used. Color pictures were taken with a Polaroid recorder on the Zeiss LSM 410 microscope.

Results

Four series of experiments were carried out. An immunocytochemical study using a monoclonal antibody against α-tubulin was undertaken to reveal the particular microtubular systems affected by nocodazole treatment. The second series of experiments was performed to see whether macronuclear infection of *P. caudatum* with *H. obtusa* was possible under nocodazole treatment. The goal of the third series was to find whether the cause of the infection block was the impaired phagocytosis. In situ hybridization experiments were carried out in the fourth series to reveal the location of *H. obtusa* in nocodazole-treated cells.

Various microtubular systems could be clearly revealed in paramecium cells by labeling with DM 1A antibody: ciliary

axonemes, basal bodies, contractile vacuole rootlets, the buccal cavity with peniculi, quadrulus and supporting curtain of microtubules, cytopharyngeal microtubules, postoral fibers, and the internal microtubular network (Fig. 1A–C). The pattern of microtubule disassembly under nocodazole treatment was exactly the same in all cells observed. The internal microtubular network radiating from the cytopharynx (Fig. 1D, E) seemed to be disrupted first, followed by the

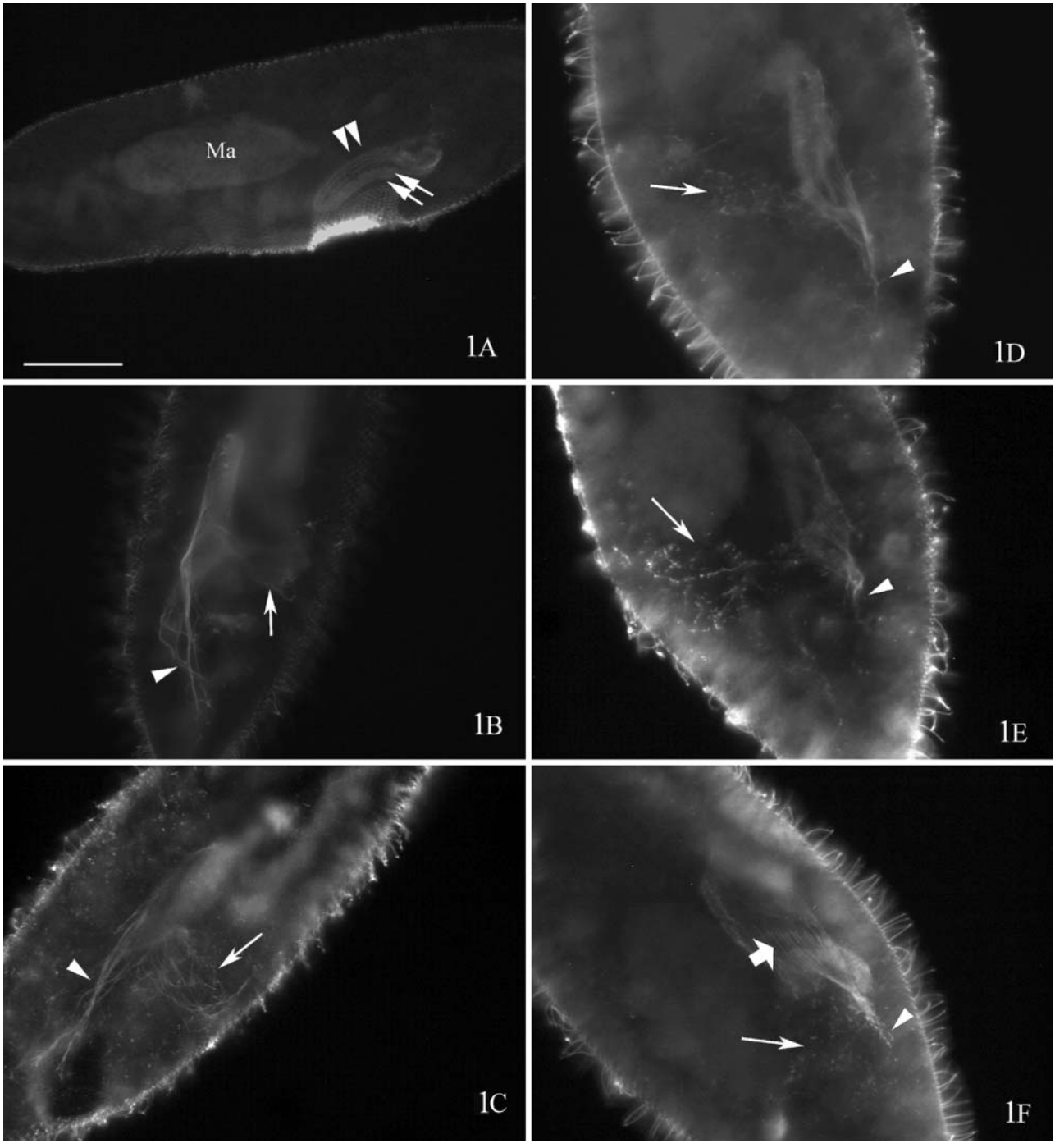

Fig. 1 A–F. Immunofluorescent labeling obtained with monoclonal antibody against α-tubulin in *P. caudatum*. **A–C** Control cells. **A** Buccal cavity in focus. Double arrows indicate peniculi; double arrowheads, quadrulus. The macronucleus (*Ma*) is revealed by counterstaining with 4′,6-diamidino-2-phenylindole. **B** Postoral fibers (arrowhead) in the focal plane. **C** Cytopharyngeal ribbons (arrow). **D–F** Nocodazole-treated cells. Note the dotted appearance of the postoral fibers (arrowheads) and cytopharyngeal ribbons (arrows) in all cells. **F** The short arrow points to the curtain of microtubules supporting the buccal cavity. Bar: 25 μm

disassembly of postoral fibers (Fig. 1F). Both the cytopharyngeal microtubules and the postoral fibers became shorter and had a dotted appearance. Short immunofluorescent dashes and dots were distinctly visible in the area formerly occupied by the extensive microtubular network arising from the cytopharynx (Fig. 1D–F). However, a curtain of mirotubules supporting the oral apparatus remained stable even under long (2 h) nocodazole incubation.

In the second series of experiments, at least 20 cells were examined in each experimental and corresponding control group. We failed to observe any *H. obtusa* in Ma under nocodazole treatment during the 2 h after homogenate addition, while infectious forms invaded Ma in the control cells.

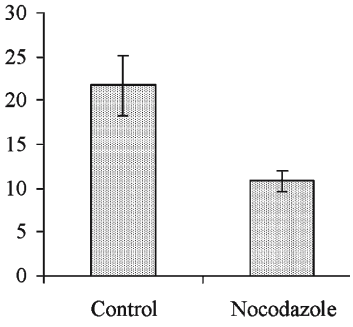

Fig. 2. Mean phagosome number in nocodazole-treated and control cells. Summarized data of 5 experiments, in each experiment at least 20 cells were examined from each (experimental and control) group. The confidence interval is 0.95. The standard deviations are indicated

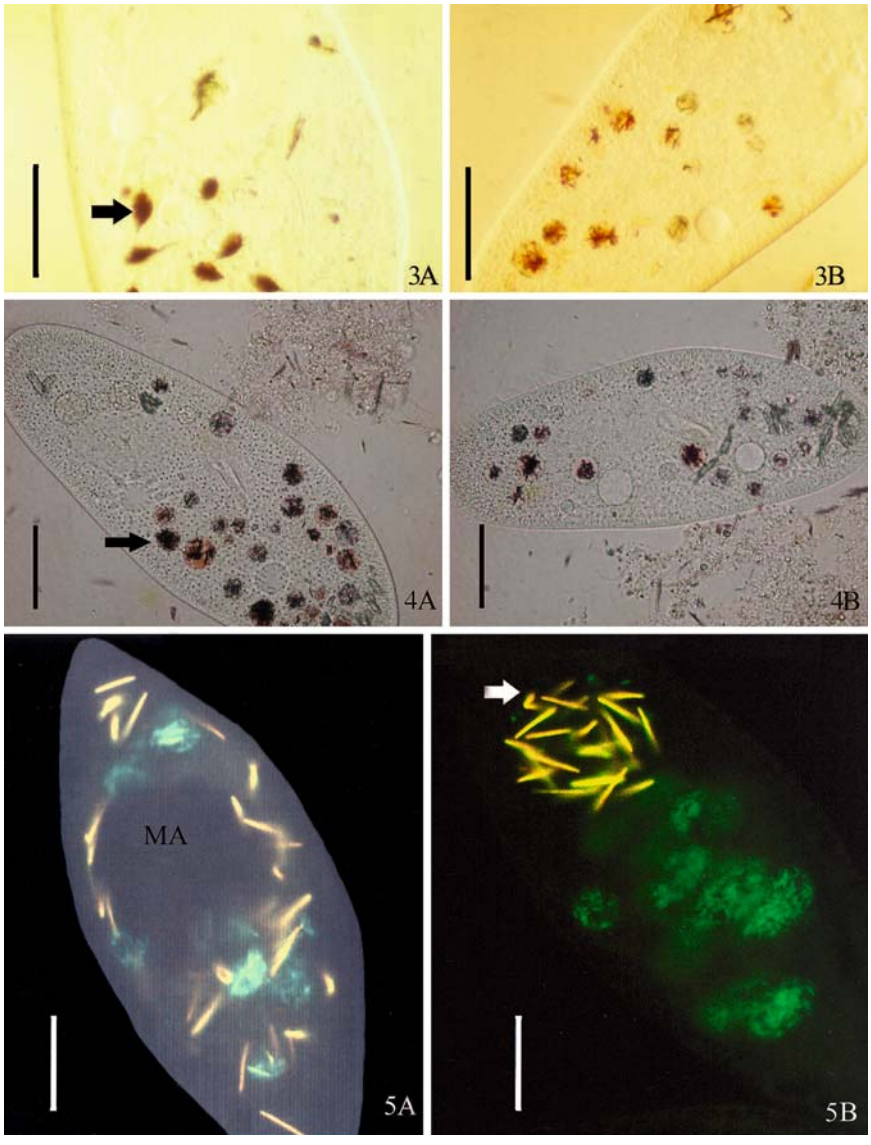

Fig. 3 A, B. Phagosomes in *P. caudatum*. Newly formed phagosomes are revealed with Kongo red. **A** Nocodazole-treated cell. Arrow indicates phagosome densely packed with prey bacteria. **B** Control cell. Note newly formed, loosely packed phagosomes. Phagosomes are distributed all over the cytoplasm. Bars: 25 μm

Fig. 4 A, B. Phagosomes in *P. caudatum*. **A** Nocodazole-treated cell. Arrow indicates posterior location of phagosomes revealed with Kongo red. **B** Control cell. Phagosomes are distributed evenly all over the cytoplasm. Bars: 25 μm

Fig. 5 A, B. In situ hybridization and confocal laser scanning microscopy. A double labeling with eubacteria- and α-subgroup-specific probes. *Holospora obtusa* are labeled with the α-subgroup probe (yellow), prey bacteria are labeled as eubacteria (green). **A** *Paramecium caudatum* infected with *H. obtusa* under nocodazole treatment. *Holospora obtusa* is not detected in the macronucleus (*MA*). **B** *Paramecium caudatum* infected with *H. obtusa* without nocodazole treatment. Arrow indicates *H. obtusa* located in the macronucleus. Bars: 25 μm

This result indicates that nocodazole does block *H. obtusa* infection of *P. caudatum*.

To make sure that the block in infection was not caused by an inability of nocodazole-treated cells to form phagosomes, the number of newly formed digestive vacuoles was counted in living cells. Prey bacteria were stained by adding Kongo red to the inoculated lettuce medium. This made it possible to distinguish "new" digestive vacuoles formed after the beginning of nocodazole treatment from "old" ones. The number of phagosomes in the nocodazole-treated cells ranged from 1 to 26, with a mean of 12. This was considerably less than their number in the control cells, which ranged from 1 to 57, with a mean of 22 (Fig. 2); however, at least one phagosome was detected in all experimental cells observed. Digestive vacuoles differed considerably in their appearance. "Dense" phagosomes, which were so tightly packed with prey bacteria that no liquid phase could be observed (Fig. 3A), were more characteristic of the nocodazole-treated cells. The more common digestive vacuoles, containing liquid along with bacteria (Fig. 3B), could be seen in both groups. The liquid content of the phagosomes stained with Kongo red was pink or blue, depending on the maturation stage of the digestive vacuole. Some small vacuoles stained with Kongo red, occurring only in the control group, did not contain any prey bacteria at all. In nocodazole-treated cells, digestive vacuoles were predominantly located in the posterior part of the cell, irrespective of the duration of the experiment (Fig. 4A). Only unstained, old phagosomes could be detected in the anterior region. In contrast, in control cells, phagosomes could be found throughout the whole cell (Fig. 4B). Thus, nocodazole did not abolish phagosome formation but significantly affected cytoplasm cyclosis.

Nomarski contrast and phase-contrast observations did not permit us to unequivocally determine the location of *H. obtusa* in the ciliate cytoplasm under nocodazole treatment. To detect the bacteria in the paramecium cytoplasm, in situ hybridization with eubacteria- and α-subgroup-specific probes was performed (Fig. 5A, B). Digestive vacuoles loaded with prey bacteria labeled with a eubacteria-specific probe (green) were scattered through the cytoplasm. It should be noted that with in situ hybridization, unlike the preparations obtained with Kongo red staining, the old phagosomes are revealed along with the digestive vacuoles formed after the beginning of the experiment, as all prey bacteria are labeled green with the eubacteria-specific probe. The infectious *H. obtusa* stages, double labeled with eubacteria- and α-subgroup-specific probes (yellow), were clearly revealed in the cytoplasm. They were located outside the digestive vacuoles, some of them having reached as far as the border of the nucleus. Thus, our data clearly show that nocodazole treatment neither prevents the escape of bacteria from the phagosome nor inhibits bacterium transport through the host cell cytoplasm, but completely blocks nuclear infection.

Discussion

The ability of intracellular endobionts to use the host cell cytoskeleton for transportation within the cytoplasm is a well-known phenomenon. Most bacteria studied so far, such as members of the genera *Legionella*, *Listeria*, *Salmonella*, *Shigella*, and *Yersinia*, usurp actin microfilaments of the host cell (Tilney and Portnoy 1989, Cossart 1995). Conversely, a growing number of observations suggest the existence of microtubule-dependent invasion systems characteristic of *Campylobacter jejuni*, *Citrobacter freundii* (Oelschlaeger et al. 1993), *Orientia tsutsugamushi* (Kim et al. 2001), and *Actinobacillus actinomycetemcomitans* (Meyer et al. 1999). At the same time, the idea of a coordinated transport by means of microtubular and microfilament systems seems highly plausible (Goode et al. 2000).

Microtubules serve as tracks for transportation of cell organelles and vesicles and are indispensable for normal Golgi functioning. In ciliates, they are considered to be involved in cytoplasm cyclosis (Cohen and Beisson 1988). In dividing *P. caudatum*, nocodazole, an agent causing depolymerization of the microtubular cytoskeleton, has been shown to affect macronuclear shape and movements, to inhibit growth of the cytospindle, and to block elongation of microtubules in the Ma and in the separation spindle of the micronucleus (Cohen and Beisson 1988). Nocodazole treatment of interphase *Paramecium tetraurelia* cells has been reported to cause disassembly of the microtubular network (Torres and Delgado 1989); however, according to these authors, the postoral fibers and the microtubule bundles associated with the buccal apparatus remained stable. Our data provide evidence for the disassembly of cytopharyngeal microtubules and postoral fibers in *P. caudatum* under nocodazole treatment, the extent of depolymerization apparently depending on the treatment duration. The cytoplasmic microtubule network radiating from the cytopharynx seemed to be the most sensitive to nocodazole treatment, which is in good agreement with the observations made by Torres and Delgado (1989) on the closely related species *P. tetraurelia*. However, postoral fibers were also destroyed in our experiments, though this appeared to take longer time.

In *P. caudatum*, a phagosome is formed as discoidal vesicles fuse with the cytopharyngeal membrane (Schroeder et al. 1990). The significant decrease in phagosome number observed in nocodazole-treated cells might be explained by the disruption of the microtubular ribbons which transport

these vesicles to the location of nascent phagosome formation. As the phagosome membrane is derived from discoidal vesicles, the lack of these might also have been the cause of dense phagosome formation. The decrease in the number of infective bacteria ingested by the cell during the course of experimental infection may have been a result of the reduction in phagosome number. Phagosome formation was not completely abolished in our experiments, possibly because of the moderate nocodazole dosage and relatively short treatment period. In nocodazole-treated cells, the phagosomes were successfully pinched off from the cytopharynx, which is in good agreement with observations made by Torres and Delgado (1989).

The posterior location of the phagosomes formed under nocodazole treatment corresponds with the data reported by Torres and Delgado (1989) and might be explained by the impairment of cytoplasm cyclosis following microtubule disintegration. This supports the idea that microtubules are involved in cytoplasm cyclosis (Cohen and Beisson 1988). Microtubules are differentially involved in facilitating different types of transport. Sokolova et al. (1998) showed that receptor-mediated endocytosis of epidermal growth factor (EGF) in epidermoid carcinoma cell cultures does not need microtubules for the delivery of EGF-receptor complexes to lysosomes. However, the transport of the fluid-phase marker horseradish peroxidase was blocked in late endosomes in the same cell line under nocodazole treatment. Thus, according to Sokolova et al. (1998), constitutive endocytosis was significantly affected by nocodazole in these cells. Nocodazole did not interfere with the internalization step in either receptor-mediated or constitutive endocytosis; however, it influenced the distribution of vesicles in the cytoplasm.

The absence of bacteria in the Ma of nocodazole-treated cells that preserve the ability to uptake both prey bacteria and *H. obtusa* demonstrates the inhibitory effect of nocodazole on *H. obtusa* invasion. The capacity of bacteria to reach the macronuclear region in the presence of the microtubule depolymerizer nocodazole provides evidence for microtubule-independent trafficking of *H. obtusa* and implies involvement of other cytoskeleton systems (probably microfilaments) in this process. On the contrary, the inability of *H. obtusa* to invade the final destination compartment, the Ma, under nocodazole treatment clearly argues for a role for microtubules in the entry of bacteria into the nucleus. Either microtubules are directly involved, or, more likely, the disturbance of cytoplasm cyclosis by microtubule disassembly interferes with some traffic pathway important for the ability of Ma to be invaded by *H. obtusa*. Constitutive endocytosis might be hampered by nocodazole in *P. caudatum*, as is the case in epidermoid carcinoma cell cultures. The entry of bacteria into the Ma is poorly understood and the observations seem contradictory. Projections of the nuclear envelope towards the transport vesicle enclosing the bacterium were revealed in electron micrographs obtained by Ossipov and Podlipaev (1977), while later observations showed that *H. obtusa* may invade the Ma not only by means of membrane fusion between the nuclear envelope and the transport vesicle but also directly, being encircled only by a fibrillar sheath at the moment of entry into the nucleus (Görtz and Wiemann 1989). It is noteworthy that microtubules are known to mediate the transport not only of membrane-bound organelles and vesicles towards the nucleus but also of free cytosolic bacteria and virus capsids (Kim et al. 2001). The immunocytochemistry results obtained in the present study do not permit any definite conclusions concerning the mechanisms of bacterial entry into the nucleus to be made. By light microscopy it is not possible to discriminate single microtubules that might assist *H. obtusa* in their entry into Ma. Intact microtubules appear to be a prerequisite for the invasion of the nucleus; however, a thorough study using EM techniques is necessary to elucidate this issue.

Our data demonstrate that nocodazole affects *H. obtusa* infection at two steps: first, at the point of bacterial entry into the cell by interfering with phagocytosis; second, at the point of bacterial entry into the nucleus. The defective cyclosis observed under nocodazole treatment does not prevent bacteria from reaching the nucleus, implying that bacteria do not use cyclosis to reach the nucleus. It is tempting to suggest that both microfibrillar and microtubular systems are involved in *H. obtusa* invasion. Microfilaments appear to be responsible for *H. obtusa* transportation during the early steps of infection, while microtubules seem to be indispensable at the later steps leading to bacterial entry into the nucleus.

Acknowledgments

We are greatly indebted to J. Beisson for her protocol for monoclonal anti-α-tubulin decoration in paramecia. Thanks are also due to the staff of the Scientific Research Center "Chromas", Biological Research Institute of Saint Petersburg State University. This work was supported by grants from the Russian Foundation for Basic Research 01-04-48550 and UR.07.01.330 to E. V. Sabaneyeva.

References

Allen R, Fok A (2000) Membrane trafficking and processing in *Paramecium*. Int Rev Cytol 198: 277–318

Amann RI, Binder BJ, Oslon RJ, Chrisholm SW, Deereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial population. Appl Environ Microbiol 56: 1219–1925

– Springer N, Ludwig W, Görtz H-D, Scheifer K-H (1991) Identification in situ and phylogeny of uncultured bacteria endosymbionts. Nature 351: 161–164

Cohen J, Beisson J (1988) The cytoskeleton. In: Görtz H-D (ed) *Paramecium*. Springer, Berlin Heidelberg New York Tokyo, pp 363–392

Cossart P (1995) Actin based bacterial motility. Curr Opin Cell Biol 7: 94–101

Fok A, Allen R (1988) The lysosome system. In: Görtz H-D (ed) *Paramecium*. Springer, Berlin Heidelberg New York Tokyo, pp 301–324

Fokin SI (1993) Bacterial endobionts of ciliates and their employment in experimental protozoology. Tsitologia 35: 59–91 (in Russian with English summary)

– (2004) Bacterial endocytobionts of ciliophora and their interactions with the host cell. Int Rev Cytol 236: 181–249

– Brigge T, Brenner J, Görtz H-D (1996) *Holospora* species infecting the nuclei of *Paramecium* appear to belong into two groups of bacteria. Eur J Protistol 32 [Suppl] 1: 19–24

– Skovorodkin IN, Sabaneyeva EV (2003) Initial steps of infection of the ciliate *Paramecium* by bacteria *Holospora* sp. Tsitologia 45: 1127–1133

Fujishima M (1996) Further study of the infectivity of *Holospora obtusa*, a macronucleus specific bacterium of the ciliate *Paramecium caudatum*. Acta Protozool 25: 345–350

– Fujita M (1985) Infection and maintenance of *Holospora obtusa*, a macronucleus-specific bacterium of the ciliate *Paramecium caudatum*. J Cell Sci 76: 179–187

– Mizobe Y (1988) Host genes controlling maintenance of a macronucleus-specific symbiont *Holospora obtusa* of *Paramecium caudatum*. Zool Sci 5: 1272

Goode BL, Drubin DG, Barbes G (2000) Functional cooperation between the microtubule and actin cytoskeletons. Curr Opin Cell Biol 12: 63–71

Görtz H-D (1986) Endonucleobiosis in ciliates. Int Rev Cytol 102: 169–213

– (1988) Endocytobiosis. In: Görtz H-D (ed) *Paramecium*. Springer, Berlin Heidelberg New York Tokyo, pp 393–405

– (1996) Symbiosis in ciliates. In: Hausmann K, Bradbury P (eds) Ciliates: cells as organisms. Gustav Fischer, Stuttgart, pp 441–462

– Wiemann M (1989) Route of infection of the bacteria *Holospora elegans* and *Holospora obtusa* into the nuclei of *Paramecium caudatum*. Eur J Protozool 24: 101–109

Kawai M, Fujishima M (2000) Invasion of the macronucleus of *Paramecium caudatum* by the bacterium *Holospora obtusa*: fates of the bacteria and timings of invasion steps. Eur J Protistol 36: 46–52

Kim S-W, Ihn K-S, Han S-H, Seong S-Y, Kim I-S, Choi M-S (2001) Microtubule- and dynein-mediated movement of *Orientia tsutsugamushi* to the microtubule organizing center. Infect Immun 69: 494–500

Meyer DH, Rose JE, Lippmann JE, Fives-Taylor PM (1999) Microtubules are associated with intracellular movement and spread of the periodontopathogen *Actinobacillus actinomycetemcomitans*. Infect Immun 67: 6518–6525

Oelschlaeger TA, Tall BD (1997) Invasion of cultured human epithelial cells by *Klebsiella pneumoniae* isolated from the urinary tract. Infect Immun 65: 2950–2958

– Guerry P, Kopecko D (1993) Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii*. Proc Natl Acad Sci USA 90: 6884–6888

Ossipov DV (1981) Problems of nuclear heteromorphism of the unicellular organisms. Nauka, Leningrad (in Russian)

– Podlipaev SA (1977) Electron microscope examinations of early stages of infection of *Paramecium caudatum* by bacterial symbionts of the macronucleus (iota-bacteria). Acta Protozool 16: 289–308

Preer LB (1969) Alpha, an infectious macronuclear symbiont of *Paramecium aurelia*. J Protozool 16: 570–578

Rautian M, Skoblo I, Lebedeva N, Ossipov D (1990) Complementation in the symbiont-host system formation between the clones of the ciliate *Paramecium caudatum* and the stocks of the bacterium *Holospora obtusa*. Tsitologia 32: 584–591 (in Russian with English summary)

Schroeder C, Allen RD, Fok AK (1990) Vesicle transport along microtubular ribbons and isolation of cytoplasmic dynein from Paramecium. J Cell Biol 111: 2553–2562

Skovorodkin IN (1990) A device for immobilization of biological objects in light microscope studies. Tsitologia 32: 301–302 (in Russian with English summary)

Sokolova IP, Arnaoutov A, Blagoveshchenskaya AD, Nikolsky NN, Kornilova ES (1998) The influence of nocodazole on epidermal growth factor (EGF) receptor endocytosis. Tsitologia 40: 855–861 (in Russian with English summary)

Tilney LG, Portnoy DA (1989) Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. J Cell Biol 109: 1597–1608

Torres A, Delgado P (1989) Effects of cold and nocodazole treatments on the microtubular systems of *Paramecium* in interphase. J Protozool 36: 113–119