Yeast-Like Endosymbionts in an Ichneumonid Wasp

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Vegetative cells and spores of a yeast-like microorganism found in various tissues in both sexes of the ichneumonid wasp *Pimpla turionellae* are passed to the offspring by infection of the oocytes. Because of their intranuclear spindles with spindle pole bodies associated with the nuclear envelope as pole structures, the microorganisms are thought to be yeasts or closely related to yeasts. The high vitality and fertility of the wasps seem to exclude a pathogenic infection. Both the passage of vesicles from the microorganisms to the host cytoplasm and their transmission to the next generation by the oocytes point to an endosymbiotic relationship.

Introduction

In the course of a study of oogenesis in *Pimpla turionellae* L. (Ichneumonidae, Hymenoptera) we noticed the presence of spores derived from yeast-like cells which can be passed to the offspring by infection of the oocyte. They are found in the hemolymph and the fat body of both sexes. In females, the growing oocytes, the follicle cells and especially the nurse cells were also infected. Circumstantial evidence indicates that we are dealing with endosymbionts rather than a pathogenic infection. Since endosymbiotic yeasts, in contrast to bacteria, are only rarely found in insects and have never been reported for hymenoptera, we thought a short notice to be in order.

Material and Methods

The wasps were reared in mesh wire cages at a daily illumination by neon lights of 16 h. The culture method is based on literature data [1, 2]. The animals were fed with a freshly prepared mixture of agar and honey [1]. Since females need an additional protein source for egg maturation, they were given the opportunity to feed on the hemolymph of host pupae. Pupae of the wax moth, *Galleria mellonella*, whose pupal cocoon had been removed, served as hosts for egg deposition. The moths were reared at 31 °C on old honey bee hives.

Wasps whose behavior indicated that they were ready for egg deposition [1], were isolated and anesthesized by putting them briefly into the ice box of a refrigerator. Then they were decapitated and the ovipositor with the attached genital apparatus was pulled out in insect Ringer. For electron microscopy, the ovarioles were fixed 2 h in 2.5% glutaraldehyde in 0.05 M collidin buffer (pH 7.2), washed in buffer, postfixed in 1% OsO_4 in the same buffer (1 h) and embedded in Epon. Ultrathin sections were stained with a saturated aqueous solution of uranyl acetate (15 min) and lead citrate (7 min). Electron micrographs were taken with a Siemens Elmiskop 101 at 80 kV. The distribution of the yeast-like symbionts in various tissues was studied with the fluorescence microscope on squash preparations stained for 30 min at 37 °C with 0.1 µg/ml aqueous solution of the fluorochrome DAPI (4′,6-diamino-2-phenyl indole) which has a high affinity for AT-rich DNA. Ethanol-acetic acid (3:1) was used as a fixative.

Results and Discussion

Fig. 1 shows one of the presumed symbiont cells in the cytoplasm of a growing oocyte. Cisternae of the endoplasmic reticulum of the host cell are closely applied to the plasmalemma of the symbiont. Since the cisternae are studded with ribosomes only at the surface facing away from the enclosed cell, the latter seems at places to be surrounded by double membranes (arrow heads). Its cytoplasm contains free ribosomes, elongated profiles of rough endoplasmic reticulum and a few microtubules. Mitochondria have never been found. The nucleus is typical of a eukaryote cell but without a single, compact nucleolus. Binucleated cells in division are shown in Fig. 2, in this case in a section through a nurse cell. The spindle is intranuclear with microtubules growing out from a dense spindle pole body associated with the nuclear envelope. Since this mode of spindle formation is quite characteristic of yeast cells [3], we assume that we are dealing with a yeast or a fungal organism closely related to yeasts. The dense spindle pole bodies, though without microtubules, are retained

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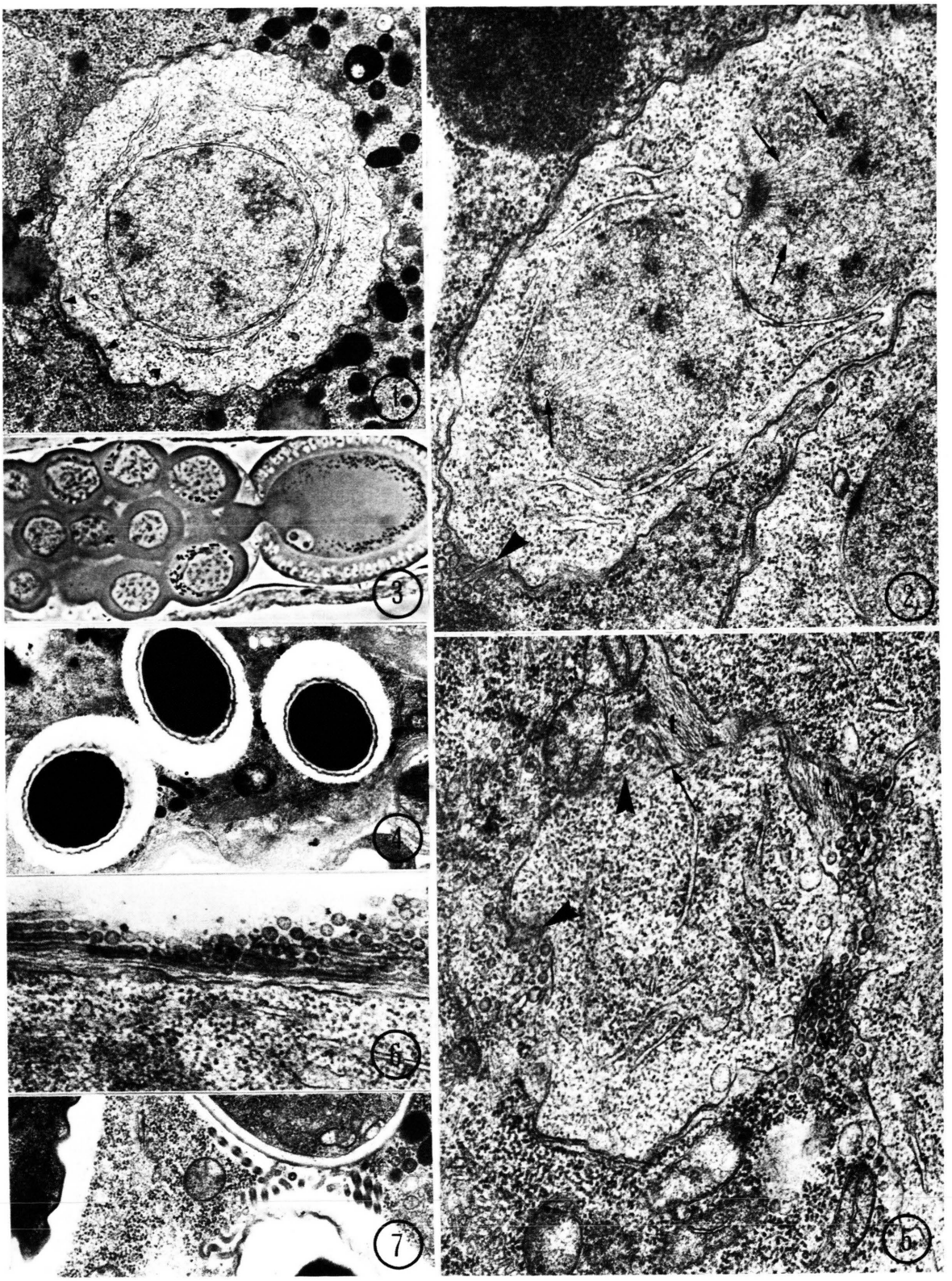
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at interphase (Fig. 2, lower right), a feature also known from yeasts [4]. Spindle pole bodies isolated from yeast cells have been shown to nucleate the formation of microtubules *in vitro* from a tubulin solution [5]. The chromosomes are connected to the spindle pole body by a microtubule ending in a dense conical kinetochore (Fig. 2), a situation also known from yeasts and lower fungi which have rather small chromosomes [6]. Conical kinetochores with single microtubules have recently also been found in the ciliate *Colpoda steinii*, again an organism with very small chromosomes. Special polar structures are absent in this case [7].

Besides vegetative cells, the cytoplasm of most tissues and the hemolymph is seen to contain spores of about 2.5 µm diameter and up to 6 µm length. Because of their high refractive index, they are readily noted in semithin sections in the phase contrast microscope (Fig. 3). In this case, they are clustered in the vicinity of the nuclei in a nurse cell chamber. In the electron microscope, they appear of uniform density and enclosed by a wall of about 25 nm thickness (Fig. 4). The spores are always surrounded by a clear zone which may arise as a consequence of the decreasing volume of the cells during the compaction of their content. The observation of intermediate stages of condensation (parts of such cells are included in Fig. 7) permits the conclusion that the spores are indeed derived from the vegetative cells described above.

The nature of the spores cannot be determined since we do not know whether the nuclear divisions are meiotic or mitotic. It is evident from Fig. 2 that binucleated cells are capable of a further division. In all cases both nuclei of a cell were in the same stage of division. The succession of two nuclear divisions within the same cell could be taken as meiosis leading to haploid ascospores. On the other hand, if the divisions are mitotic, the spores might be persistent diploid chlamydospores.

Two types of structures found only in the immediate vicinity of the yeast-like cells are indicative of a close relationship between host and symbiont. One of these is represented by an accumulation of vesicles (v, Fig. 5) of about 50 nm diameter which may, at least in part, arise from more slender (25 nm) tubules of the type arrowed in Fig. 5 and shown at higher magnification in Fig. 6. Close inspection of a number of electron micrographs shows that these tubules may be so densely packed that the impression of wavy microfilaments (t, Fig. 5) arises. Whenever these vesicles and tubules are present, the plasmalemma of the yeast-like cells seems interrupted at places (arrow heads, Fig. 2, 5). Since true membrane discontinuities are most unlikely, this must mean the existence of many outfoldings within the thickness of the section. The vesicles and tubules observed are thus given off by the yeast-like cells into the cytoplasm of the host cell. The second type of special structure, found close to some of the spores, consists of 60 nm tubules whose dense wall has about the same thickness (35 nm) as the outer dense layer of the spore wall itself (Fig. 7). However, direct connections between the two structures have never been observed. We are unable to make any specific statement regarding their function.

Intracellular yeast-like organisms have to our knowledge never been reported for hymenoptera.

Fig. 1. Symbiont cell in a young oocyte. Endoplasmic reticulum of the host cell is so closely applied to the plasmalemma that it appears doubled (arrow heads). ×19 500.

Fig. 2. Binucleated cell in mitosis or meiosis. In the upper nucleus, microtubules (arrows) radiate from a dense spindle pole body to the chromosomes, the upper arrow pointing to a kinetochore. The nucleus below is also in division as seen by the dense chromosomes and the radiating microtubules (arrow); the spindle pole body is out of the plane of sectioning. At the lower right, part of a nucleus of a binucleated cell in interphase is shown. The spindle pole body is present without microtubules. The arrow head at the left points to vesicles which seem to arise from the symbiont cell. ×32 000.

Fig. 3. Semithin section, phase contrast. Note the accumulation of dense spores close to some of the large nurse cell nuclei. The nurse cell chamber is connected to the cytoplasm of the oocyte (right) which is surrounded by the follicular epithelium. The oocyte nucleus contains the small proteinaceous endobody and the chromatin in the larger caryosphere. The granules in the egg cytoplasm are yolk. ×170.

Fig. 4. Spores in the connective tissue surrounding the follicle cells. ×10 500.

Fig. 5. Vegetative cell surrounded by vesicles (v) and densely packed tubules (t). A single tubule is arrowed. The cell membrane appears interrupted at the arrow heads (comp. also Fig. 2). ×32 000.

Fig. 6. Vesicles seem to be pinched off from slender tubules arranged parallel to the cell membrane of the symbiont. ×48 000.

Fig. 7. Tubular structures in the vicinity of spores. Two of these (above and left) are in stages of progressive condensation. The irregular contour of the cell at the far left is presumably due to artificial shrinkage. ×21 300.

While bacterial endosymbionts are frequently found in insects [6], intracellular yeasts are rare. Yeasts of the *Candida*-type have been found in coccids [7], cerambycid beetles [8–10] and some grashopper species [11, 12]. In the case of the beetles whose larval food consists of wood, it is assumed that the yeasts complement the protein poor diet [8, 10]. It has been shown that the host receives amino acids and vitamins of the B-complex and that isolated symbionts are capable of the assimilation of urea, ammonium, uric acid, amino acids and peptones. This led the authors to the assumption that the yeast utilizes waste products of its host and supplies it in turn with amino acids and vitamins. In the grashoppers, the symbionts were predominately in the fat body of adults of both sexes. In the female, they are passed through the follicular epithelium into the posterior pole of the oocyte. The ball of symbionts which accumulates there is thus passed to the next generation. Experiments showed that the host dies before reaching adulthood if the yeasts are killed by exposure to a temperature above 35 °C.

In *Pimpla*, fluorescence microscopy of DAPI-stained tissues showed the presence of the microorganisms especially in the fat body and the hemolymph of both sexes. As in the grashopper, the ovarian tissues including the egg cells themselves are infected. In the early stages of oogenesis, most of the presumed yeast cells, both vegetative stages and spores, are found in the large endopolyploid nurse cells (Fig. 3). As in all insects with polytrophmeroistic ovarioles the nurse cell chamber has an open connection to the ooplasm. Towards the end of oogenesis, the whole cytoplasm of the nurse cells streams into the oocyte. This should be the pathway by which most of the yeast-like cells reach the egg to be passed to the next generation. Their final location is in clefts of the oosome, a specialized cytoplasmic region at the posterior pole which normally gives rise to the germ cells. There is no indication that we are dealing with a pathogenic infection in our stock of wasps since its vitality and fertility corresponds to published data on the life cycle of *Pimpla turionellae* [2]. Instead, both the close cytological interrelationship between the microorganisms and the host cells described above and the passage to the offspring point to symbiosis. At the present, we have no indication regarding the metabolic interrelationship between the host and its presumed symbionts. As the wasps normally thrive on a diet of honey which is poor in protein, a role in the reutilization of nitrogeneous wastes as in the case of the cerambycid beetles mentioned above [8] seems not unlikely.

In a thorough light microscope study of the oogenesis of *Pimpla turionellae* based on paraffin sections, Meng [15] described inclusions of 4–5 µm diameter of high contrast surrounded by a light halo. She found them in very young stages in the vicinity of the egg nucleus and in mature eggs within the oosome. She called these bodies chromidia because of their dark staining with histochemical reagents for protein. Although she could not find a positive Feulgen reaction for DNA, the chromidia seem morphologically identical with the spores of the yeast-like microorganisms described in this paper. The reason for the negative Feulgen reaction may be due to the prolonged fixation (5–24 h) in Bouin's fluid followed by 15 min hydrolysis in N HCl at 60 °C since the picric acid in the fixative leads to a slow hydrolysis of DNA. Even under optimal conditions the Feulgen reaction is so weak that we preferred a fluorochrome. Another discrepancy between Meng's findings and ours is the low number [1–3] of chromidia. This may be due to the use of stained paraffin sections (5–7 µm) since it has been our experience that the spores are readily noticed only in thinner sections viewed unstained with the phase contrast microscope. She mentions indeed in connection with the chromidia the presence of numerous other granules and platelets at the oocyte envelope. However, we cannot exclude that Meng's stock might have contained considerably less symbionts or that proportionately more cells might have been in the vegetative state since only the spores are readily recognizable by light microscopic methods.

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Erratum

G. Laskay, T. Farkas, E. Lehoczki, and K. Gulya, Effects of Pyridazinone Herbicides during Chloroplast Development in Detached Barley Leaves. II. Effects on Lipid Content, Fatty Acid Composition, and Ultrastructure of Chloroplasts, Z. Naturforsch. **38c,** 741–747 (1983).

Unfortunately the legend of Table I of this article is incorrect, because a wrong unit of measure was given.

The correct version is:

Table I. Amounts (μg/g fresh weight) of various fatty acids and lipids from chloroplasts of barley leaves after 72 h of greening in the presence and absence of pyridazone herbicides.

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