Virus-like Particles and Rickettsia-like Organisms in Male Germ and Cyst Cells ofBemisia tabaci(Homoptera, Aleyrodidae)

Sônia N. Báo
Elliot W. Kitajima
Giuliano Callaini
Romano Dallai

Insect sperms have attracted intensive attention from the cytologists, especially those trying to correlate the large diversity of their structure and the evolutionary process. We recently engaged in a study of male germ cell differentiation and structure in whiteflies, a less studied though important group of insects.

During our study it became apparent that male germ and cyst cells of Bemisia tabaci contain rickettsia-like organisms and also virus-like particles. This note describes details of these findings. In a separate article a more comprehensive description of B. tabaci sperm is being made.

Adult males of B. tabaci were kindly supplied by Dr. Josias C. Farias, from his colony maintained on soybean at Centro Nacional de Pesquisa do Arroz e Feija˜ o (Goianˆ ia, GO, Brazil). Whiteflies were dissected and the testes fixed for 1 hr in 2.5% glutaraldehyde, 1.8% sucrose in a 0.1 M phosphate buffer and then for 1 hr in 1% osmium tetroxide in the same buffer. The samples were dehydrated with acetone and then embedded in Epon resin. After sectioning and staining with uranyl acetate and lead citrate, the sections were examined with a JEOL 100 C or Philips CM 10 transmission electron microscopes.

Virus-like particles (VLP). VLPs were detected in four of six of the examined male B. tabaci. These particles were found in both germ and cyst cells (Figs. 1–4). They were present in the nucleus of spermatids and spermatozoa, but in the cyst cells, VLPs were noted only in the cytoplasm. In the nucleus of young spermatids, VLPs were always found interspersed in the dense, compacted chromatin region (Fig. 1). However, as the differentiation process continued, they were found dispersed in the less dense area (Fig. 2). Finally, in more condensed nuclei in well-differentiated sperms, VLPs appeared aggregated, commonly in the central region (Fig. 3). When detected, VLPs were present in practically all spermatids and sperms, numbering up to 2 dozen/section. Similar particles were also noticed in large numbers in the cyst cells, within huge vesicles interpreted as lysosomes (Fig. 4) mixed with myelin-like structures. VLPs, however, were not observed free in the cytoplasm. The VLPs were rounded, with an electron-dense core of 20– 25 nm, surrounded by an electron-lucent zone, possibly the coat protein, 15–20 nm wide. Center-to-center distance in a regular package of the particles was 45– 50 nm.

Another remarkable finding was the presence of numerous rickettsia-like procarionts, also both in the germ and cyst cells. In the germ cells these bacterioids were localized in the cytoplasm. In early spermatids the rickettsia-like organisms appeared scattered throughout the cytoplasm (Fig. 5). As usual, as the spermatid elongated, excess cytoplasm containing the bacterioids was eliminated as residual body (Fig. 6); thus, in mature sperm they could not be found. These symbionts were also present in the cytoplasm of cyst cells (Fig. 7). In sections, they exhibited a profile that varied from round (ca. 1 mm in diameter) to elongated (2–2.5 mm long) and surrounded by a double membrane: the outer, adjacent to the host cytoplasm, thick and dense and the inner membrane, thinner and less dense. In about 5% of these symbionts, besides ribosomes and DNA, fibrillar particles, 6–7 nm thick and ca. 0.5 mm long, could be detected. They appeared in a parallel array, which in cross sections revealed an ordered, paracrystalline arrangement (Figs. 8 and 9).

Many reports have been made of VLPs in several insect groups, without being associated with a particular pathogenic condition, and are believed to be latent infections. The presence of VLPs in germ cells, however, has been less frequent, and the best interpretation is that this indicated a route of vertical transmission of the putative virus (e.g., Diptera, Schankel and Schwalm, 1975; Afzelius et al., 1989, and Coleoptera, Kitajima et al., 1985). Rickettsia-like organisms in insects have been commonly been found may be considered as a rule, and though also being detected in some few cases in male germ cells (Wright et al., 1978; Wright and Barr, 1980; Ndiaye and Mattei, 1993), their vertical transmission probably is transovarial.

In the present case, VLPs were only detected in the nucleus of the germ cells, initially interspersed in the chromatin, but as the nuclei condensed they are expelled to the nucleoplasm, finally being packed within the condensed chromatin and possibly being transmitted vertically after fertilization. In the cyst cells, VLPs are only found within lysosome-like vesicles, in huge numbers. They are possibly remnants of degraded sperm, endocyted by cyst cells. Most of the presumed latent infection by VLPs in insects described in the literature involves reovirus or picorna-type particles (Kim, 1980; Matthews, 1982; Kitajima, 1989). Those found in the present study are somewhat smaller and do not fit into any of the known insect virus families.

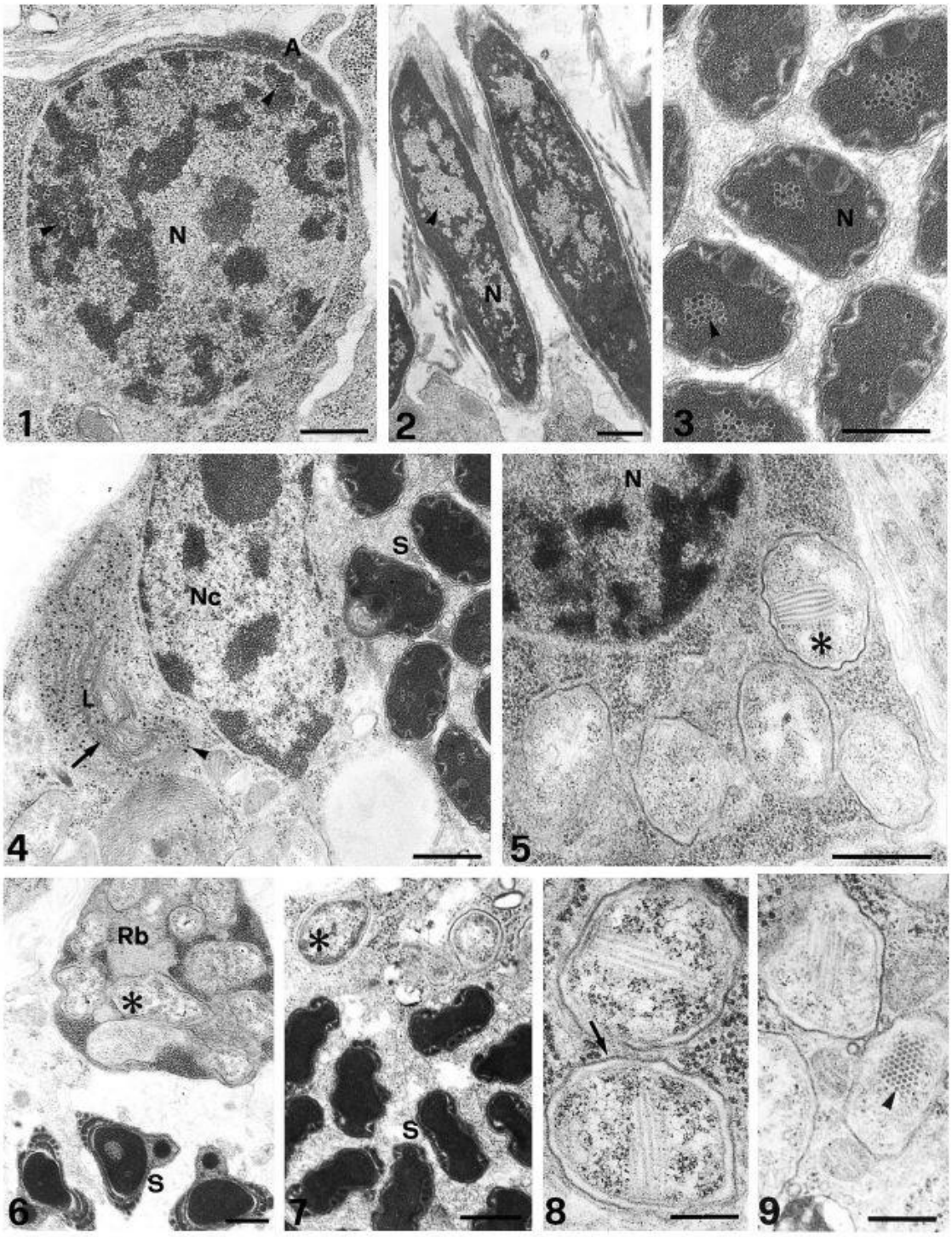

FIG. 1. Cross section from spermatid. Virus-like particles (arrowheads) are present in the electron-dense areas of the nucleus (N). A, acrosome. FIG. 2. Longitudinal section of the nucleus (N) from a spermatid. Several virus-like particles (arrowhead) appear in the less dense chromatin. FIG. 3. Cross section of the nucleus (N) from spermatozoa. Several virus-like particles (arrowhead) appear packed in the central region. FIG. 4. Cyst cell from a cyst in which spermiogenesis is almost complete. Cytoplasm contains huge lysosomes (L) with a large mass of virus-like particles (arrowhead) interspersed with myelin-like structures (arrow). Nc, nucleus cyst cell; S, spermatozoa. FIG. 5. Section of spermatid containing rickettsia-like organisms (asterisk) in the cytoplasm, none of them containing filamentous particles. N, nucleus. FIG. 6. Section of a region of the testis showing spermatozoa (S) and several rickettsia-like organisms (asterisk) in the residual bodies (Rb). FIG. 7. Rickettsia-like organisms (asterisk) can be seen in

the cytoplasm of the cyst cell. S, spermatozoa. FIGS. 8 and 9. Rickettsia-like organisms showing double membrane (arrow) and a paracrystalline array of filamentous particles (arrowhead). Bars, 0.5 mm.

The bacteria found both in germ and cyst cells have a double-layered thin envelope and are contained in a cytoplasmic vacuole similar to rickettsia-like microorganisms described in germ cells of mosquitoes. Rickettsia-like microorganisms are obligatory intracellular bacteria widespread among insects. They have been implicated as causes of parthenogenesis in Hymenoptera (Stouthamer et al., 1993) and cytoplasmic incompatibility in Homoptera, Coleoptera, Hymenoptera, Lepidoptera, and Diptera (for review, see Rousset and Raymond, 1991). We do not know if this is also the case in Bemisia, where these intracellular bacteria are found for the first time. They seem unnecessary to sperm function because they are eliminated. The filamentous particles found within them morphologically resemble phages of the Inoviridae family and may be responsible for eventual degeneration of the Rickettsiae as reported in mosquitoes (Yen, 1976; Ndiaye and Mattei, 1993).

We conclude that the virus-like particles and rickettsia-like microorganisms represent agents that infect the B. tabaci testes, but they apparently do not interfere in the spermiogenesis process.

KEY WORDS: Rickettsia-like; Bemisia tabaci; viruslike particles; whiteflies; insect sperm; male germ cell.

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