Ultrastructure of New Viruslike Particles in Drosophila

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Three kinds of viruslike particles have been found in various organs during an ultrastructural investigation in Drosophila melanogaster. While one of these particles, about 40 nm in size, appears identical to similar particles already described, the two others (one of them intranuclear, about 50 nm; the other one intracytoplasmic, about 60 nm) are described for the first time in Drosophila. The relationships with other insect particles described in the literature are discussed.

INTRODUCTION

Viruslike particles have repeatedly been found since 1964 in several different tissues of various Drosophila stocks (Philpott et al., 1969; Rae and Green, 1968; Akai et al., 1967; Filshie et al., 1967; Kernaghan et al., 1964). So far the findings appear to be restricted to D. melanogaster with only one exception where particles were reported in D. virilis (Akai et al., 1967), but this species was used as a host for transplantable tissues containing viruslike particles of D. melanogaster. The particles were found both in larval and in adult animals, so far only in resting cells, and they appeared to be located either in the nucleus or in the cytoplasm, or in both areas. In any instance, they appeared doughnut-shaped, maybe slightly oval, with an outer dense layer, and a transparent core. Their overall diameter, in calibrated pictures, was estimated between 38 and 42 nm (Philpott et al., 1969; Rae and Green, 1968). It seems possible that the particles so far described are of a unique kind.

The present report, in addition to one kind of particle about 40 nm similar to those reported in the aforementioned literature, describes two new different kinds of viruslike particles found in various tissues of larval as well as adult D. melanogaster. All these particles were discovered accidentally in the course of a study on the ultrastructure of different organs in Drosophila. Their relationship to the other viruslike and virus particles found in insects is discussed.

MATERIALS AND METHODS

Two stocks of D. melanogaster were used: a wild type, the Canton S, and a brachy mutant with genotype g ty bb; Y bb- (Ritossa, 1968).

For electron microscopy, larval testes, larval fat tissue, and adult ejaculatory ducts were fixed with 2% or 3% glutaraldehyde in 0.066 M phosphate buffer, pH 7.4. The osmolarity of the two fixatives was 450 and 625 millimols, respectively. After fixation for 60-120 min, the tissues were postfixed with 1% OsO4 in 0.1 M phosphate buffer, dehydrated, and embedded in Epon. Ultrathin sections, cut with either diamond or glass knives on a LKB III or a Reichert OME2 ultramicrotome, were stained with uranyl acetate and lead (either citrate or hydroxide), and observed with a JEOL JEM 120 or a Siemens Elmkspor IA electron microscope. The magnifications of these instruments were calibrated with a grating replica having 2160 lines per millimeter.

RESULTS

First Type—60-nm Particles

The 60-nm particles were found in the cytoplasm as well as in extracellular position (among the cells) of terminal cells of
larval testes, both in wild type and in bobbed mutant animals (Figs. 1–4). The particles located in the extracellular spaces were either single, or in a few units, while those located within the cytoplasmic matrix were either isolated or in clusters. When in clusters, they exhibited a regular crystalline arrangement, suggesting regular geometric properties on their outer surface (Figs. 2, 3). The particles were observed in contact with free ribosomes or with cell membranes, although no obvious relationship was noticed with these cell components. They were about 60 nm in size, showing a polygonal—frequently hexagonal—outline; they consisted of a dense outer layer, about 75 Å thick, which surrounded a comparatively less dense core, about 45 nm in diameter (Fig. 4). The central core frequently showed coarse condensations of a dense material (Figs. 3 and 4) which may be artifactual. The outer layer, under suitable orientations, exhibited a sharp outline (Fig. 4). The geometric properties of the outer layer suggest a nucleocapsidic structure, with an outer capsid made of orderly arranged capsomeres, like several “true” viruses of insects and other organisms.

Second Type—50-nm Particles

A second kind of particle was observed inside the nucleus of larval fat body cells in the bobbed mutant (Figs. 5–7); they were not observed within the cytoplasm. The particles were scattered in the nuclear area, either isolated or collected in clusters, but no regular crystalline arrangement was observed (Fig. 5). They were sometimes observed in close contact with the nucleolar material (Fig. 6). Each particle was about 50 nm in size, doughnut-shaped, and exhibited a dense outer layer surrounding a comparatively less dense central core. The latter, about 27 nm in diameter, was in the form of amorphous material of variable density (Fig. 7). The outer layer was about 110 Å thick, more dense towards the interior, granular or spiky in structure, and ending with a fuzzy outline.

Third Type—40-nm Particles

These particles, which are similar to the 38–42 nm particles reported in the literature, were observed in a tracheolar cell lying in the hemocoel, in close contact with the basement membrane of the muscular cell surrounding the ejaculatory duct (Figs. 8, 9). They were located only inside the nucleus, collected in a disordered cluster, showing no particular relationship with the nuclear components. Each particle was doughnut-shaped, about 40 nm in size, showing a dense outer layer surrounding a comparatively less dense, centric core (Fig. 8). The latter, about 25 nm in diameter, was in form of amorphous material of variable density; the outer layer was about 70 Å thick (Fig. 9).

Discussion

The particles from Drosophila described herein are of three different kinds, varying in size, shape, and intracellular location. They have been seldom and haphazardly discovered, in the course of studies on the fine structure of various Drosophila tissues, when attention was not going to be devoted primarily to them. Since their discovery, no experiments have been undertaken yet to determine their nature, so that only an attempt to classify them on morphological grounds is possible.

There is a general agreement that particles like these are not normal components of the cells. Furthermore, even in the lack of infectivity tests, there is a strong suspicion that they are viral in nature, because they are morphologically similar to other particles whose viral nature has been ascertained.

All the viruslike particles in Drosophila described so far in the literature appear to be round, maybe slightly oval, doughnut-shaped, around 38–42 nm in size (lower and
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Fig. 1. Intracytoplasmic viruslike particles, about 60 nm, in terminal cells of Drosophila melanogaster larval testis: bobbed mutant animal. BM, basement membrane. × 20,000.

Fig. 2. Intracytoplasmic particles in a wild-type animal. The particles show crystalline arrangement. IC, interstitial cells. × 20,000.

Fig. 3. Enlarged detail of Fig. 1: the cluster of viruslike particles is surrounded by free ribosomes. × 60,000.
Fig. 4. High magnification of the particles. The sharp outline of the outer coat is visible under suitable orientations (arrows). × 200,000.

Fig. 5. Intranuclear viruslike particles, about 50 nm, in an adipose cell of fat tissue: bobbed mutant larva. L, space devoid of lipid (removed during specimen preparation); G, glycogen; n, nucleolus. × 20,000.
Fig. 6. Enlarged detail of Fig. 5. The particles lie in the nuclear matrix, some of them in close contact with the nucleolar material. n, nucleolus; chr, chromatin material; R, ribosomes; G, glycogen. × 60,000.

Fig. 7. High magnification of the particles. The dense layer that limits the core (arrows) and the spikes of the outer coat (short arrow) are visible. × 200,000.
Fig. 8. Intranuclear viruslike particles, about 40 nm, in a tracheolar cell of a bobbed mutant imago. chr, chromatin material. × 40,000.

Fig. 9. High magnification of the particles. × 200,000.
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higher figures being attributable to different technical conditions and to lack of calibration), able to crystallize, usually intranuclear (Philpott et al., 1969; Rae and Green, 1968; Akai et al., 1967; Filshie et al., 1967; Kernaghan et al., 1964), but also intracytoplasmic (Akai et al., 1967). The 40 nm particles found within the nucleus of tracheolar cells fall into this group of particles.

Viruslike particles of this size have been detected in other insect species: Aedes aegypti, among the Diptera (Filshie et al., 1967); Antheraea eucalypti (Filshie et al., 1967), and Hyalophora cecropia (Peters and Staal, 1968), among the Lepidoptera.

Other particles of this size have been described in other insect species, all of them Lepidoptera: Antheraea pernyi, Hyalophora cecropia, Actias selene, Philosamia cynthia × ricini (Longworth and Harrap, 1968), and Gnomota podocarpi (Harrap et al., 1966). These particles are the nonoccluded virions of true viruses which are responsible for diseases that affect the larval stages of their insect hosts. The fact that apparently there are among the members of this dimensional class of 40 nm both DNA and RNA viruses, since the virus of Philosamia cynthia × ricini has been described as a DNA-virus (Longworth and Harrap, 1968), and that of Gnomota podocarpi as a RNA-virus (Harrap et al., 1966), expresses the limits of a classification on morphological basis, and stresses the need of additional data to pinpoint the taxonomic position of all these insect particles.

Turning to the Drosophila viruslike particles described in this paper, it appears that both the 50 nm and the 60 nm particles are shown for the first time in Drosophila. The intranuclear, 50 nm particles, merely on the basis of their size and shape, may be compared only with viral particles of an unnamed larval disease of midgut in the Lepidopteran Antheraea eucalypti (Grace and Mercer, 1965). These particles, observed under negative contrast, were round, about 500 Å in size, and doughnut-shaped.

The 60 nm intracytoplasmic particles can be compared only with morphologically similar viruslike particles found in images of the Hymenopteran Formica lugubris (Steiger et al., 1969). Here the comparison is consistent, since in both instances the particles, observed in this sections, were spherical with polygonal outline, able to crystallize, and showed the same intracytoplasmic location.

It seems rather interesting that these particles were found in the same tissue, i.e., the terminal cells of the larval testes of members of different stocks of Drosophila, but the significance of this coincidence is not clear. It will be interesting to search for the presence of such particles in the same tissue of other strains of Drosophila.

REFERENCES


