REGULARLY ARRANGED PARTICLES ON THE INNER LAMELLA OF THE PLASMA MEMBRANE OF *ACANTHAMOEBA PALESTINENSIS*

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INTRODUCTION

Recently, it was suggested that portions of the plasma membrane of *Acanthamoeba palestinensis* are composed of curved pieces of membrane or plaques, which may be derived from and/or give rise to cuplike cytoplasmic vesicles (1). This paper, previously reported in abstract form (2), further delineates the mosaic nature of the plasma membrane of this cell by describing the existence of regions of the plasmalemma where regularly arranged particles are attached to the cytoplasmic surface.

METHODS

Cultures of *A. palestinensis*, *A. castellanii*, and *A. rhysodes* were grown in axenic suspensions in proteose peptone–glucose medium as described by Band (3, 4). Fractions enriched with plasma membranes were isolated as described previously (1), resuspended in 0.25 M sucrose–0.001 M disodium ethylenediaminetetraacetic acid (EDTA), homogenized with three strokes of a 10 ml Potter-Elvehjem glass Teflon grinder at 1000 rpm, and washed three times by centrifugation at 1100 *g*. For electron microscopy, cells were fixed in a growth medium diluted with 5 vol of 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 hr at room temperature (5). The cells were rinsed in five changes of cold buffer over a 2 hr period, postfixed in cold Zetterqvist's (6) osmium solution for 45 min, rinsed in H_2O, dehydrated through a graded series of ethanol to propylene oxide, and embedded in Epon (7). The cells were pelleted by low-speed centrifugation and resuspended by gentle pipetting during solution changes. Plasma membrane fractions were fixed and dehydrated as described above, being processed as pellets in centrifuge tubes. Following dehydration, the pellets were broken into halves and embedded in Epon, oriented so that the plane of sectioning included the entire thickness of the pellet. Thin silver sections were cut using a Porter-Blum MT-1 ultramicrotome, placed on grids coated with 0.3% parlodion, and stained with 4% aqueous uranyl acetate and Reynolds' (8) lead citrate. Sections were viewed with a Philips 300 electron microscope.

RESULTS

The most common configuration of the free surface of *A. palestinensis* is shown in Fig. 1. The trilaminar unit membrane structure of the plasmalemma was about 100 A thick and asymmetrical, with the outer, electron-opaque leaflet being thicker and more densely stained than the leaflet adjacent to the cytoplasm (5). The cell surface was frequently characterized by a rippled appearance caused by alternating furrows and curved pieces, or plaques, with their concave surfaces facing the cytoplasm. The range in length of the plaques (~60–370 nm) corresponded to the length of cuplike cytoplasmic vesicles which have been suggested to give rise to and/or pinch off from the cell surface (1).

Some patches of the plasma membrane were observed to have a coat on their inner surface. At low magnifications these regions were detectable by their unusually high electron opacity (Fig. 2) and at higher magnifications a layer of particles oriented perpendicular to the plasma membrane was revealed (Figs. 3, 4, and 5). The length of the particle-studded patches of plasmalemma varied

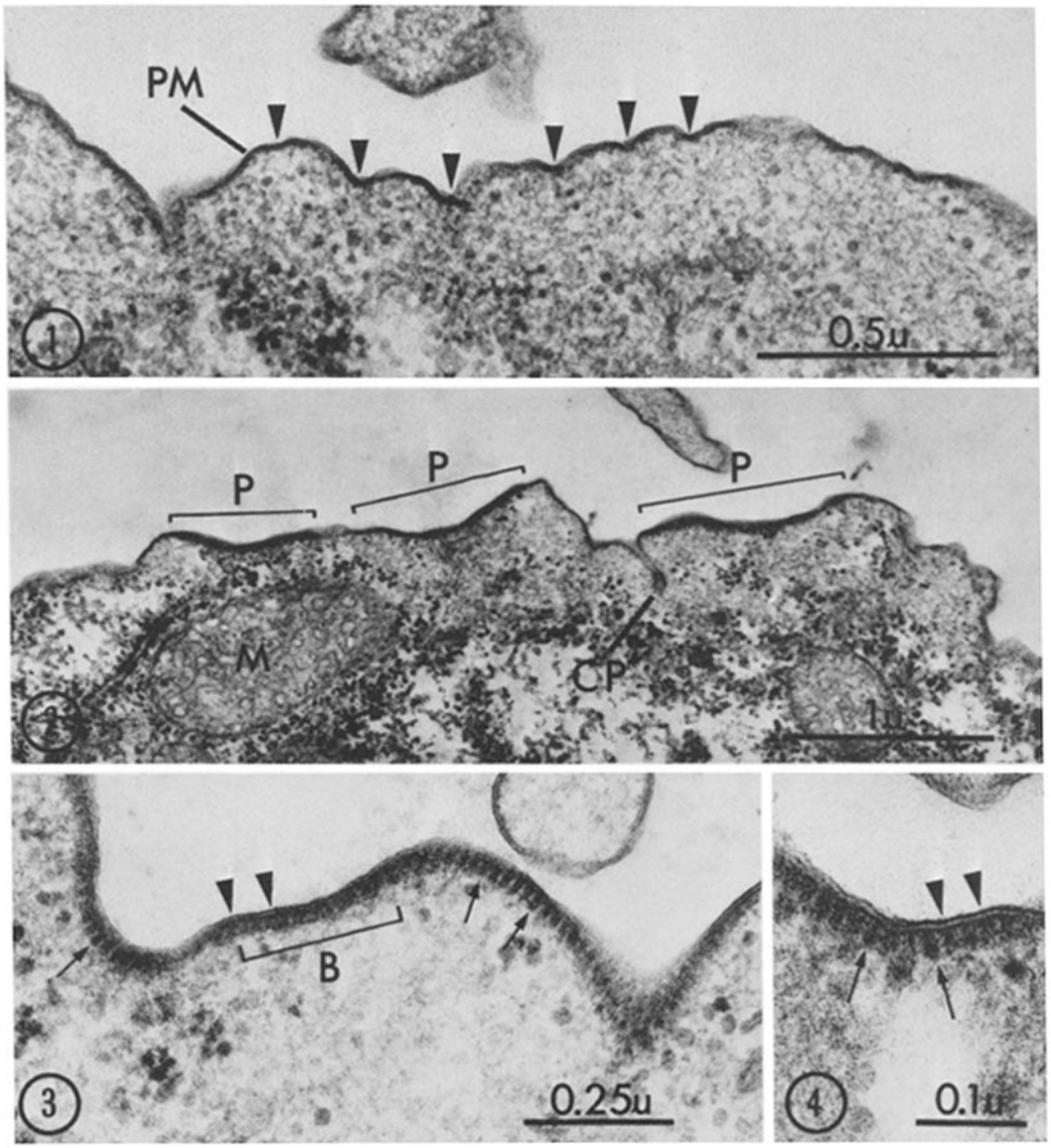

FIGURE 1 An electron micrograph through the periphery of *Acanthamoeba palestinensis*. The unit membrane of the plasmalemma (*PM*) is visible, with its thicker or more densely stained outer leaflet. Certain areas of the plasma membrane appear to be made up of plaquelike structures separated by furrows (arrowheads). × 64,000.

FIGURE 2 An electron micrograph similar to that of Fig. 1, except that the plasma membrane has three patches (*P*) which appear thick and electron opaque. The thickened areas of the cell surface show little curvature, while an invagination with a coated pit (*CP*) can be observed on an unthickened region of the plasmalemma. (*M*), mitochondria. × 28,000.

FIGURE 3 In this electron micrograph of the cell surface, the particulate coat appears blurred in one area (*B*) and periodic in others. Particle-coated areas of membrane can be seen to have relatively straight, convex, or concave surfaces facing the cytoplasm. The unit membrane structure is clearly visible in some regions (arrowheads). The arrows point to particles whose structure seems apparent. × 80,000.

FIGURE 4 An electron micrograph of a small, particle-covered area of the plasma membrane. The particles seem to be attached to the cytoplasmic lamella of the plasma membrane which is slightly convex in this image. The structure of some of the particles (arrows) seems to be visible (see text for description). Notice that the structure of the middle and outer leaflets of the unit membrane appear to be unaffected by the attachment of the particles to the cytoplasmic leaflet (arrowheads). × 147,000.

from about 0.3 to 1.9 μ. Such patches of membrane were observed alone (Figs. 4 and 6) in the plane of section or in small groups (Figs. 2, 3, and 5).

In some areas the layer was visualized as a blurred coating, while in other areas its periodic nature was evident (Fig. 3). This indicated that the particles were arrayed in rows, and that the thickness of the sections included several superimposed rows of particles. This was confirmed in tangential sections, which illustrated the particles arranged in rows (Figs. 5 and 6). In all tangential sections examined, the particles appeared to be in hexagonal arrays. In those areas where only a small portion of the length of the particles had been retained in lightly grazing tangential sections, they seemed to be composed of a light core surrounded by a dark, circular wall (Fig. 5).

When viewed at an angle normal to the plasmalemma, the uniform, longitudinal profiles of the particles appeared to be about 250 A long, 160 A in diameter, and were periodically arranged with a center-to-center spacing of ~220 A. Some images (Figs. 3, 4, and 5) indicated that the particles were like tubes with slightly bulbous free tips—their bases being continuous with the cytoplasmic leaflet of the plasma membrane. Thus the particles appeared as short hollow cylinders both in longitudinal and in cross section. The electron-opaque wall of some of the particles seemed to be coated with barely discernible, fine projections. The electron-transparent middle leaflet and the thick, electron-opaque outer leaflet of the plasma membrane were not visibly altered by the presence of the particles (Figs. 3 and 4).

The particulate coat was visualized on areas of the plasmalemma bent at various angles (Figs. 2, 3, 4, and 5), indicating that the presence of the particles did not seriously hinder the flexibility of the membrane. This, coupled with the observation that the distance between the free tips of the particles was greater on portions of the cell surface presenting a convex face toward the cytoplasm than on straight or concave surfaces (Figs. 3 and 5), indicated that cross-linking did not occur between particles over their entire length. However, given the precise periodic arrangement of the particles, it is possible that cross-linking occurred at their site of attachment to the inner leaflet of the plasmalemma.

Although vesiclelike structures with the particulate coat sometimes were observed underlying the cell surface (Fig. 5), these probably represented infoldings of the plasmalemma since they were seen only near the cell surface. An infolding of the plasma membrane which, if sectioned appropriately, could have given rise to such vesiclelike structures is shown in Fig. 5.

The structure of the particles and their relationship to the plasma membrane were preserved in isolated membranes (Fig. 7). Thus, the attachments of the particles to the membrane were fairly stable, since these associations remained intact during the isolation procedure, despite homogenizations and washings of the membranes in 0.25 M sucrose–0.001 M EDTA. Similar particles were not observed on plasma membranes of fixed cells or on isolated membranes of *A. castellanii* and *A. rhysodes*, which had been grown under conditions identical to those for *A. palestinensis*.

DISCUSSION

The functional significance of the periodically arrayed particles protruding from the inner lamella of the plasma membrane of *A. palestinensis* is not known. These particles appear similar to those of "coated pits" seen in these cells (Fig. 2) and previously described by Bowers and Korn (5) in Neff's *A. castellanii*, except that the particulate coat of such pits was localized to a very small area at the base of invaginations of the plasma membrane (Fig. 2). Such coated pits looked similar to the forming micropinocytotic vesicles described in a variety of cell types (9, 10, 11, 12, 13, 14, 15). In contrast, the large particles described in this paper apparently were not associated with cytoplasmic vesicles or with any specific curvatures of the plasma membrane. For these reasons, the particles do not seem to be specifically associated with vesicle or vacuole formation.

Except for their larger dimensions, the membrane-associated particles in *A. palestinensis* are similar to shorter, periodically arranged particles reported to line extensive areas of cell surface membranes involved in ion transport in insect cells (16, 17, 18, 19, 20, 21). In epithelial cells of the rectal papillae of the blowfly, *Calliphora erythrocephala*, arrays of ~150 A long particles were reported to cover the inner leaflet of the highly infolded luminal plasma membrane (16, 17). Anderson and Harvey (18) demonstrated the existence of similarly arranged, ~125 A long particles on the plasma membrane delimiting mitochondrial-containing cytoplasmic projections of the goblet cells in the larval midgut of the silkworm,

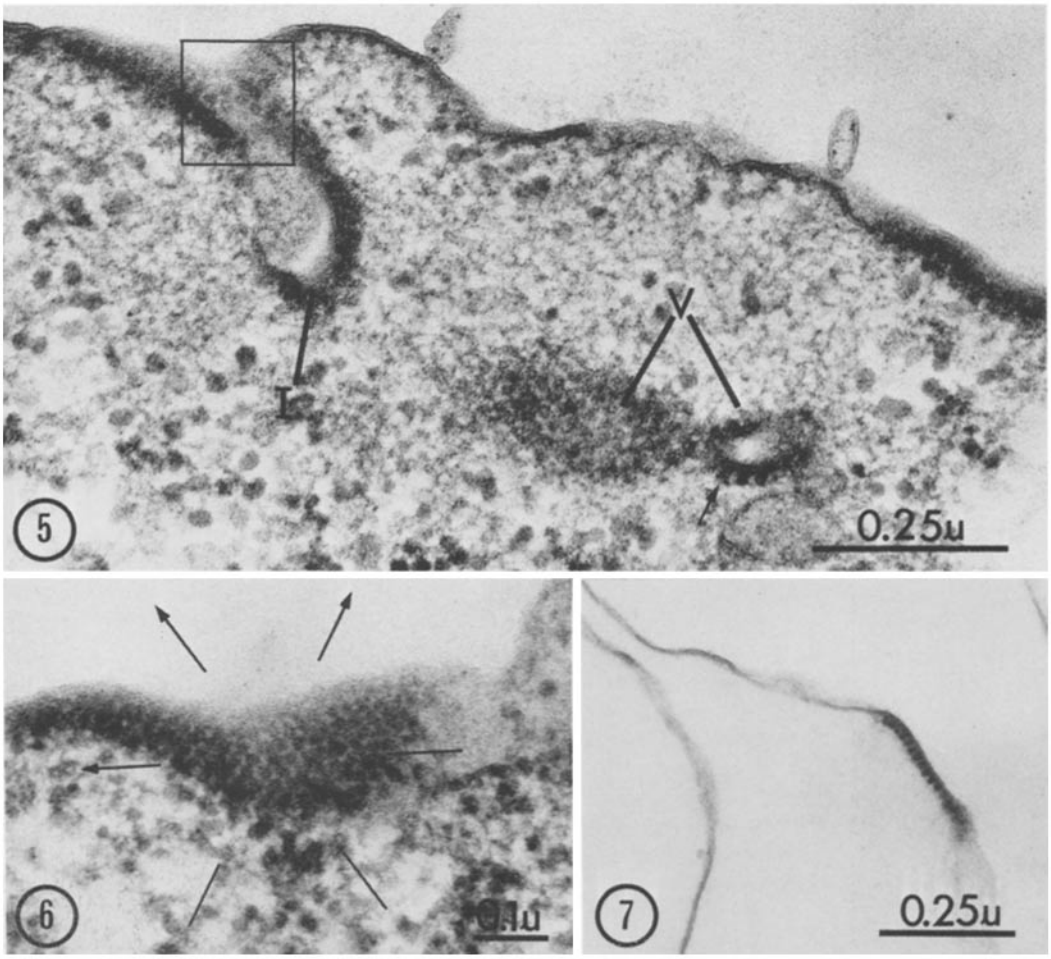

FIGURE 5 Particle-covered vesiclelike structures (*V*) and an infolded piece (*I*) of plasma membrane can be observed. Hexagonally arranged particles can be seen in tangentially sectioned areas and a light core in some of the tangentially sectioned particles is seen in the square. The structure of a longitudinal profile of a particle seems to be apparent on a highly convex surface of membrane (arrow). × 104,000.

FIGURE 6 The rows of particles are clearly visible in this tangential section. The arrows indicate the direction of the rows. × 102,000.

FIGURE 7 A section through a pellet of isolated plasma membranes. A segment of plasma membrane covered with particles identical to those observed *in situ* can be seen. × 60,500.

Hyalophora cecropia. Such particles also were reported on the plasma membrane of goblet cells in the larval midgut of *Ephestia kuhniella* (21), as well as in the Malpighian tubules responsible for urine secretion in other insects (19, 20).

An ouabain-insensitive transport of potassium has been demonstrated in *C. erythrocephala* rectal papillae cells (17), as well as in the midgut of *H. cecropia* (22, 23). Therefore, it is possible that the particles were the sites of the enzymes mediating this function. However, a magnesium-dependent adenosine triphosphatase was localized by electron microscope histochemistry only on the lateral membranes of *C. erythracephala* rectal papillae cells (17) and not on the particle-studded apical membranes. In light of this, Gupta and Berridge (16, 17) postulated that the particles on the apical membranes may represent "complex enzyme systems generating energy for transport processes." Anderson and Harvey (18) have suggested that a

ouabain-insensitive mitochondrial ion accumulation is important in the goblet cells of *H. cecropia* midgut, and that the particles also may function to allow the sizable potassium transport known to occur in the midgut (22).

While more work is needed to establish the role of the membrane particles in *A. palestinensis*, it is noteworthy that, as in rectal papillae cells of insects and the midgut of moths, the transport of potassium in a similar soil amoeba, *Acanthamoeba* sp., was reported not to be inhibited by ouabain (24). Thus, the particles may represent the morphological manifestation of a unique type of transport system in those cells where potassium transport is not linked to a sodium pump. Alternatively, since the related soil amoeba, *A. castellanii* and *A. rhysodes*, exhibited no such particulate arrays, the particles observed in *A. palestinensis* may only represent a unique geometrical arrangement of some common cellular component.

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