AN ATYPICAL CRISTA RESEMBLING A "TIGHT JUNCTION" IN BEAN ROOT MITOCHONDRIA

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ABSTRACT

The conformation and structure of an atypical crista found in a small percentage of the mitochondria in root tip cells of *Phaseolus vulgaris* L. have been studied electron microscopically in material fixed in glutaraldehyde followed by osmium tetroxide. In its transformation into an atypical crista, a normal crista elongates, broadens, and flattens, and the inner leaflets of its apposed unit membranes appear to fuse in a manner analogous to the formation of "tight junctions" between certain animal cells. The result is a large platelike, quintuple-layered structure, 240–260 A thick, whose long axis parallels that of the mitochondrion. The outer layers of the "plate," bordering on the mitochondrial matrix, are thickened and exhibit striking patterns in the micrographs. The structure of the plate is compared with that previously described for tight junctions between animal cells.

INTRODUCTION

The "tight junction" is one of a number of types of intercellular connections found in animal tissues. It appears to be formed by a fusion of the apposed outer leaflets of the adjacent unit membranes to result in a characteristic quintuple-layered structure. Tight junctions have been observed in a number of animal tissues, including muscle (4) and nerve (9), where they may function in the transmission of stimuli (6). They have also been reported between epidermal cells in the skin of the frog (3) and between cortical epithelial cells in rectal papillae of the blowfly (1). Using infiltration with lanthanum and uranium salts, Revel and Karnovsky (7) have shown that not all tight junctions are formed by fusion of the outer leaflets; in some, the leaflets are intact and separated from each other by a 20 A gap.

Robertson (8), in a study of the tight junctions forming the synaptic discs of the Mauthner cells in goldfish brains, has established the existence of hexagonally packed units in the apparently fused outer layers of the two unit membranes, that is, in the middle layer of the quintuple-layered tight junctions. Similar units have been observed by Revel and Karnovsky (7) in the intercellular junctions of mouse liver and heart cells. Such observations may provide an important insight into the structure of membranes in general.

The present paper describes a broad, flat, platelike crista which develops from a normal crista in a small percentage of bean root mitochondria. The basis of the atypical structure lies in an apparent fusion of the apposed leaflets of the crista membranes to form a quintuple-layered structure resembling that observed in tight junctions between animal cells. Such structures have not been reported previously in mitochondria, nor indeed have they yet been reported for any of the membranes of plant cells.

MATERIALS AND METHODS

Seeds of bean (*Phaseolus vulgaris* L. var. Dwarf Horticulture) were obtained from Olds Seed Co., Madison, Wis. Following germination the plants were grown under greenhouse conditions for 3 wk in a modified Hoagland solution. Iron as ferric tartrate was added as needed to prevent chlorosis. Some seeds were also grown for 3 days or for 1 wk in vermiculite. Tips 2–3 mm long were cut from both main and lateral roots and fixed at room temperature for 1.5 hr in 3% glutaraldehyde containing 0.025 M phosphate buffer at pH 6.8. Alternatively, some root tips were fixed in 10% acrolein in distilled water for 16 hr at 4°C. The root tips were then washed for 1 hr in four changes of 0.025 M phosphate buffer and postfixed in 2% osmium tetroxide in buffer of the same molarity for 2 hr. They were then dehydrated in an acetone series and embedded in Araldite-Epon. Sections were cut on a Sorvall MT-1 ultramicrotome with a diamond knife and mounted on copper grids (400-mesh or 300 × 75-mesh). The sections were then stained with aqueous 2% uranyl acetate followed by lead citrate and viewed in a Hitachi HU-11A microscope at 50 kv with a 30 μ objective aperture.

Mitochondria containing atypical cristae were also examined with a Hitachi HU-11 microscope fitted with the HK-2BH model tilting stage, and a series of micrographs was taken of each atypical crista in the normal untilted position and at 5° or 10° tilt and 10° or 20° increments of the azimuth position (tilt axis).

OBSERVATIONS

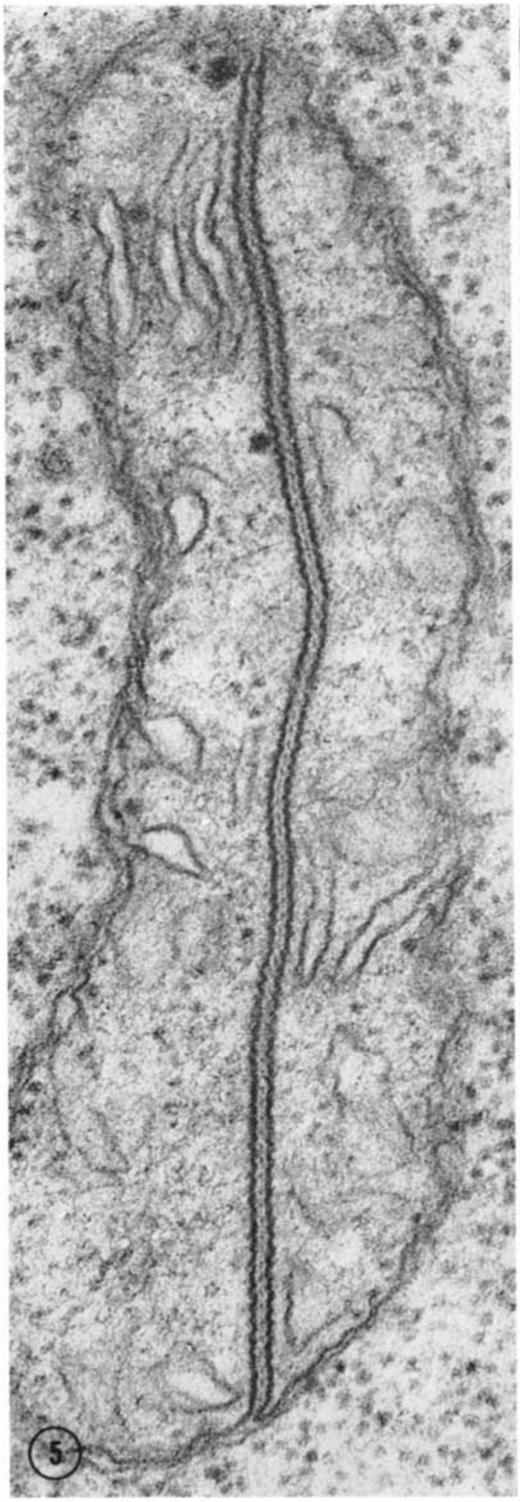
An investigation of the fine structure of bean root tips has revealed that certain mitochondria contain modified or "atypical" cristae. Evidence for the shape and extent of these cristae has been obtained from several sets of successive sections. One of these sets is represented in Figs. 1–4, which illustrate the first, second, fifth, and seventh sections of a series of seven recorded successive longitudinal sections through the same mitochondrion. Since the mitochondrion is appreciably shorter in the first and seventh sections than in the intermediate ones, the major portion of it is probably represented in the series. The two atypical cristae in the organelle have been sectioned almost normally. Although they appear rodlike, the successive sections reveal that they are nearly flat and extend through much of the mitochondrion. The crista on the left is connected to the inner mitochondrial membrane at the bottom in Figs. 1–3, whereas that on the right is connected to this membrane at the top in all four figures. The opposite ends of both cristae terminate within the matrix in characteristic pouchlike structures which resemble those of ordinary cristae.

These mitochondria have been observed in root tips from plants which were grown under several culture conditions and sampled at intervals from seed germination to the young, 3-wk-old plant. The structure of the atypical cristae is similar in material fixed in two different aldehyde fixatives, glutaraldehyde and acrolein. Although plastids are nearly as numerous as mitochondria in cells of the bean root tip and although their inner membranes are also invaginated, an alteration similar to that observed in the cristae has never been seen (5). These observations suggest that the atypical cristae are not fixation artifacts but are true structural components of some of the mitochondria in the bean root. Mitochondria with atypical cristae of similar appearance have also been observed in soybean, *Glycine max* (L.) Merr.

The exceptional mitochondria appear to be widely distributed within the root tip but occur with low frequency (probably between 0.1 and 1.0%). They have been observed in cells of the meristematic region and quiescent center, in differentiating phloem and pericycle cells, and in young root cap cells. Insufficient observations have been made to determine whether they occur in other regions as well. The exceptional mitochondria are identifiable only by the presence of an atypical crista; no other differences in fine structure are observed.

A unique structure results from the fusion and specialization of membranes in the development of an atypical crista. In its transformation a normal crista elongates, straightens, and grows laterally to form a large flattened sac, the major axis of which coincides approximately with the long axis of the mitochondrion (Figs. 1–6). Figs. 7 and 9 show examples of developing cristae; in each case an atypical crista in a formative stage is seen alongside a fully formed one. The latter stages of the alteration are accompanied by a remarkable change in the conformation of the membranes of the crista; the membranes on opposite sides come together so that their apposed inner layers either fuse or cohere, resulting in a structure similar to that of the "close connections" or tight junctions formed between certain membrane pairs in animal cells. The intracristal space is thereby completely occluded, and the crista acquires the appearance of a rigid, platelike structure with

FIGURES 1–4 Longitudinal sections through a mitochondrion containing two plates sectioned almost normally. These are the first, second, fifth, and seventh sections in a series of seven recorded successive sections. × 69,000.



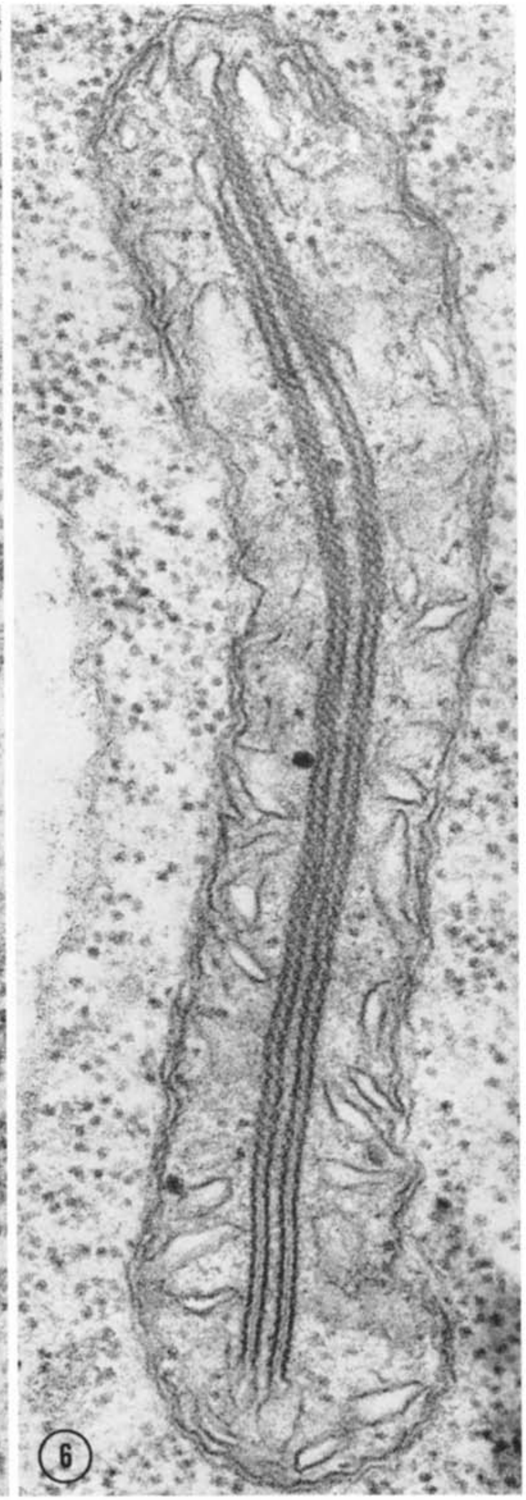


FIGURE 5 Nearly normal section of a plate confluent with the inner mitochondrial membranes at the ends. This figure clearly illustrates the striking electron opacity of the scalloped outer layers of the plate membranes. The quintuple-layered structure of the plate is evident. × 100,000.

FIGURE 6 Oblique sections through two atypical cristae. The oblique lines running across the plates are quite prominent. At the bottom the plates are normal with respect to the plane of section. × 85,000.

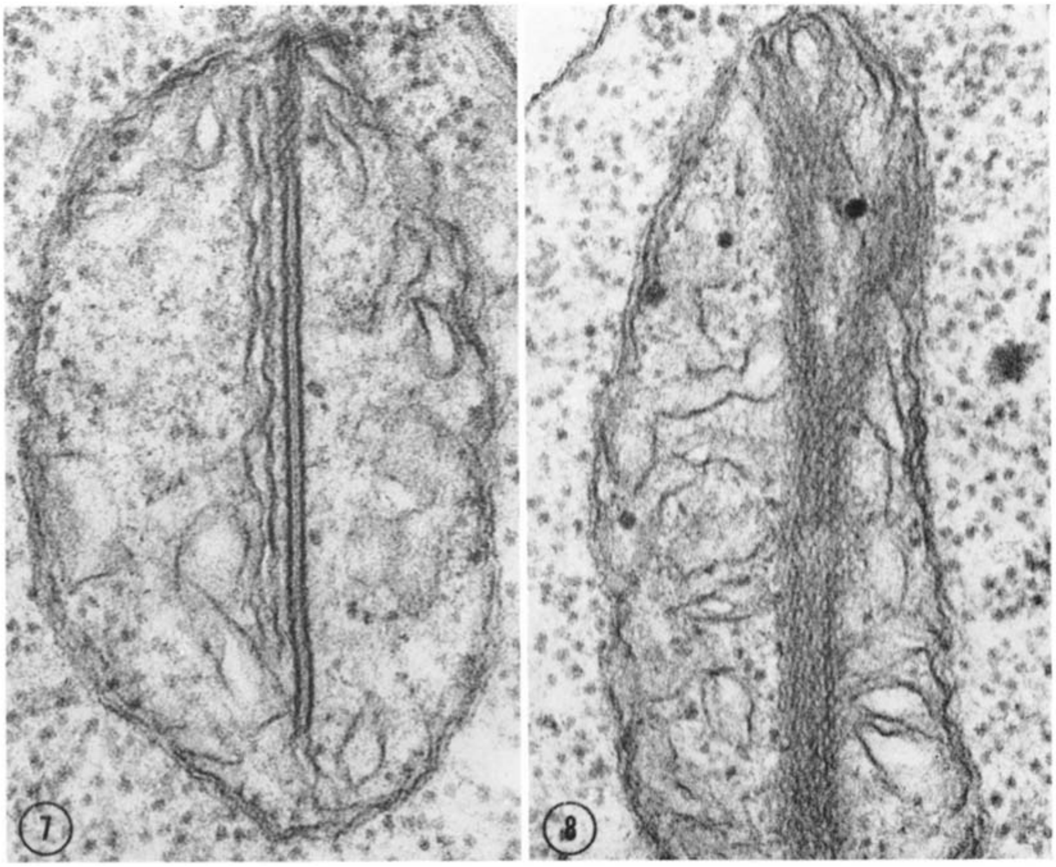


FIGURE 7 A plate is shown sectioned normally except at the upper end. The quintuple-layered structure of the plate is seen quite clearly. A second plate appears to be developing parallel to the first. × 100,000.

FIGURE 8 An approximately tangential view of a plate showing the surface pattern. The plate is apparently curved, passing into and out of the plane of section. The pattern is distorted by the curvature of the plate. × 100,000.

parallel sides (Figs. 5–7 and 9). For convenience and brevity, this structure will be termed a "plate." Fusion or coherence of the inner layers of the two membranes is accompanied by a thickening and specialization of the outer layers bordering on the mitochondria matrix (Figs. 5–7 and 9).

There is no evidence that the plates partition the mitochondria completely. For example, although the plate in Fig. 5 is continuous with the inner membrane of the mitochondrion at both ends, it is known from successive sections that this particular plate does not completely partition the organelle. Some well-developed plates do not appear to be joined to either end of the mitochondrion (Fig. 6), although presumably they are confluent with the inner membrane of the envelope in some other region.

Thin sections would be expected to reveal profiles of mitochondria sectioned both longitudinally and transversely. We have assumed that elongate profiles represent longitudinal sections (e.g. see Figs. 1–6) and that nearly circular profiles represent transverse sections (e.g. see Fig. 9) of mitochondria. The various patterns produced by the sectioning of the plate are common to both of these planes of section through the mitochondria; no association of a specific pattern with one or the other of these planes of section has been observed.

In normal or almost normal sections the appearance of the plate suggests a rather rigid struc-

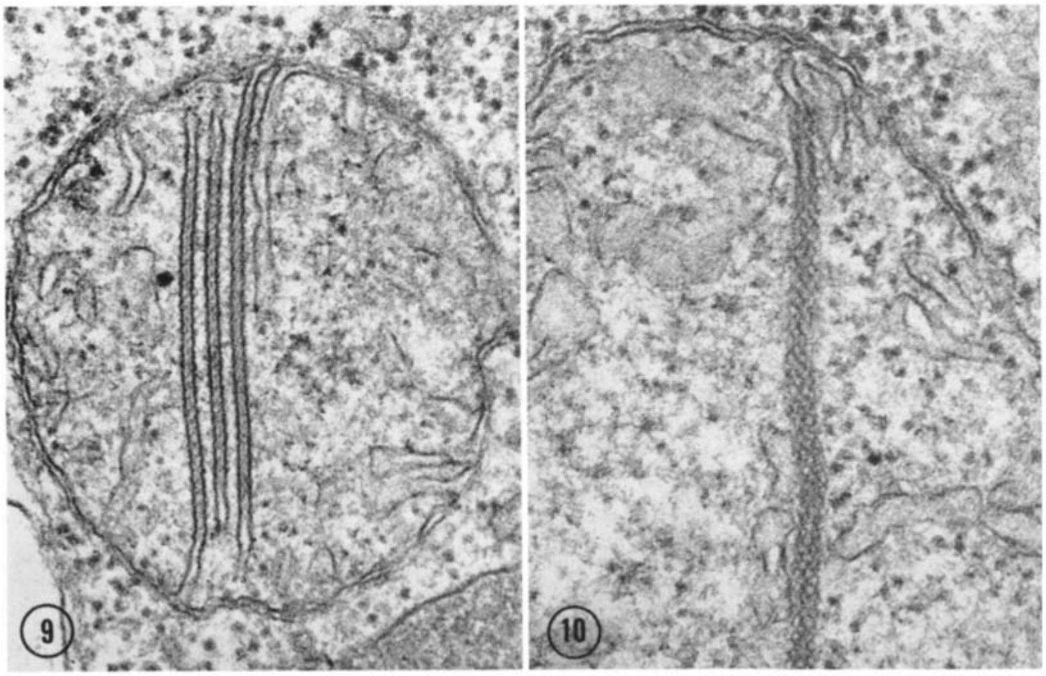


FIGURE 9 Transverse section of a mitochondrion showing three plates sectioned almost normally. To the right lies an atypical crista in an early stage of development. The plate on the right shows a clear continuity with the inner membrane of the envelope. × 80,000.

FIGURE 10 A nearly tangential section of a plate showing diamond-shaped patterns. × 100,000.

ture (Figs. 1–7 and 9). Seen in profile, the plate in some cases runs linearly through the matrix (Figs. 1–4, 7, and 9), whereas in others it curves or bends somewhat stiffly (Figs. 5 and 6). In Fig. 6, the plates curve in conformity to the curvature of the mitochondrion, remaining closely parallel even through small changes of direction.

As mentioned above, in some of the mitochondria a second crista appears to be developing into a plate parallel to the first. This process may be repeated, resulting in the formation of three (Fig. 9) or, rarely, four plates in a single mitochondrion. All plates in a given mitochondrial section have approximately the same alignment and membrane pattern, indicating a similar orientation (Figs. 1–4, 6, and 9). As further evidence for this, when a plate is seen in approximately tangential section (Fig. 8), additional plates with other orientations are never observed in that section of the mitochondrion.

Normal sections of the plate (Fig. 7) clearly reveal a quintuple-layered structure similar to that found in other reported cases of membrane fusion. The highly electron-opaque outer layers in contact with the mitochondrial matrix, each 70–85 A thick, are separated by a region 90–100 A across. This region contains two electron-lucent layers, each about 35 A thick, and a faint, electron-opaque median layer about 25 A thick. The transparent layers presumably represent the lipid components of unit membranes, and the median layer represents the fused, or possibly coherent inner layers of these membranes. The median layer may have a periodicity of structure, evident as a beaded appearance in normal sections (Fig. 7). The total measured width of the plate is 240–260 A. A clear continuity between the plate and the inner mitochondrial membrane is frequently observed (Figs. 5 and 9), which supports the interpretation that the plate arises by alteration of a crista.

The existence of an unusual three-dimensional structure in the thickened outer layers of the plate membranes is suggested by the scalloped or notched appearance of these layers when the plate is at a slight angle to the normal (Figs. 5 and 9). Study of nearly normal sections provides, in fact, a means of systematizing the seemingly wide variety of patterns obtained for the plate, since in a particular section the tilt of the plate with respect to the plane of section does not remain constant, and it is possible to follow the change in pattern along the plate as the plate departs progressively farther from normal.

Nearly normal sections exhibit patterns of two types which are assumed to represent different aspects of the same structure arising from variations in the tilt of the long axis of the plate with respect to the plane of section. One type appears as a series of curved lines in each outer layer, resembling a vertical row of figure eights (Figs. 2 and 3). Further tilting from normal gives rise to the commonly observed diamond-shaped patterns (Fig. 10). The other type is characterized by parallel oblique lines running across the plate (Fig. 6). In the latter type, the scalloped appearance of a nearly normal region is succeeded, as the tilt from normal increases, by oblique lines projecting from each outer layer. These lines progressively become more prominent and appear continuous across the plate when the tilt becomes sufficiently large (Figs. 5 and 6). Still further tilting from normal results in the appearance of diamond-shaped patterns (Fig. 7, top).

These relationships between the patterns were confirmed by the use of a tilting stage. A particular plate was found to give rise successively to the expected patterns as its angle to the viewing axis was altered, and, considered collectively, the plates exhibited all of the common pattern transitions.

The above-mentioned parallel lines so prominent in oblique sections of the plates are always oblique, never transverse (Fig. 6). They are 40–50 A in diameter and 400–500 A long, and they run across the membranes at an angle of 35°–55° to the plate axis. The distance between adjacent parallel lines, measured along the plate axis, is 120–170 A. Each line on one outer layer matches one on the other, so that when the plate is sectioned at sufficient tilt each matched pair appears as a single continuous line crossing the plate obliquely.

Sections of the plate that approach the tangential, that is, sections more or less parallel to the plane of the plate (Fig. 8), show surface views of the structure in the outer layers. The electron-opaque lines, 30–50 A in diameter, form a network which, by analogy with the structure described for a tight junction, is assumed to define a system of units 90–130 A in diameter. Since section thickness is probably at least twice the thickness of the plate, in many cases both membranes will be included in the tangential sections, resulting in a more complex pattern. This may account for the somewhat confused pattern in Fig. 8.

DISCUSSION

The similarities in structure and mode of formation between the atypical cristae herein described and the tight junctions of animal cells are particularly interesting in view of the different types of membranes and functions involved. The presence of a quintuple-layered structure formed through fusion of the outer layers of adjacent plasma membranes was demonstrated in the club endings of Mauthner cell synapses in the goldfish brain by Robertson (8). The quintuple-layered mitochondrial plate described here appears to result from a similar apposition and possible fusion; in this case it necessarily involves the inner layers of the membranes of the cristae, although this has not been demonstrated directly since tripartite structure is not ordinarily observed in membranes of the bean root mitochondria with the fixation employed.

In a study of synaptic disc structure, Robertson (8) compared the effects of different fixatives and noted that with osmium tetroxide the outer layers of the discs were quite electron opaque, whereas the fused inner layers were faint. The reverse was found with potassium permanganate, the inner layer becoming the dominant electron-opaque layer (see Figs. 4 and 6 in reference 8). The latter layer, furthermore, was shown to contain hexagonally packed units which gave rise to a hexagonal pattern in tangential sections and to a series of transverse densities in oblique sections. Revel and Karnovsky (7), using aldehyde-osmium tetroxide fixation accompanied by infiltration with lanthanum, have shown similar structures in tight junctions between mouse heart cells.

In the mitochondrial plate, the system of units is located in the outer layers and gives rise to a series of oblique densities in oblique sections. Thus, despite the over-all similarity, there are two differences between the structure of the mitochondrial plate and that of the tight junctions. One involves the layers in which visible substructure is localized, and the other involves the configuration of this substructure. Also, the over-all thickness of the mitochondrial plate is somewhat greater than that of a tight junction. However, this is due to the greater thickness of the outer layers only. The three middle layers are similar in dimensions to those of the tight junction.

There are several reports of tight junctions and close connections in which transverse densities consistent with the presence of hexagonally packed structures have been demonstrated (2, 3, 6–8). However, parallel oblique lines have never been found. These lines appear to be unique to the surface layers of the mitochondrial plates and must reflect the presence in these layers of a unique substructure.

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