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Phase Contrast and Electron Microscopic Observations on Membranous Organelles in Myxomycete Plasmodia[1]

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Abstract: Phase contrast and electron microscopic studies of the plasmodia of the phaneroplasmodium, aphanoplasmodium, and protoplasmodium types showed the presence of the usual organelles found in the cytoplasm of cells of higher organisms. Mitochondria are of the tubular type, there are both smooth and rough surfaced endoplasmic reticulum, Golgi membranes, vesicles and vacuoles of various types. Fibrils and filaments were seen in the cytoplasm, and there is a cell membrane of the usual type. Ribosomes and polysomes are seen in the ground plasm. No evidence of a centriole was ever seen.

As modern techniques of investigation are applied to an increasing number of cell types, it becomes evident that one of the most outstanding features that all cells have in common is the presence within them of membranes. Although they vary in their measurements and in the details of their appearance, probably depending on current activity of the cell, all the membranes seem quite comparable in their structure. There is increasing evidence that would support a concept of the cell as a three-phase system: *(1)* the contents of nucleus and cytoplasm linked through the nuclear pores, *(2)* the membrane system, continuous throughout the cell at least intermittently, and *(3)* the contents of the membrane-bounded channels, continuous with the environment at least intermittently (1).

This picture has emerged from the accumulation of evidence produced by studies of a great variety of plant and animal species, and increasing refinement of techniques permits a rather high degree of confidence in the concept of a number of cell organelles.

There is little reported work, using the newer techniques. on the myxomycetes, a group of organisms belonging to the Protists, intermediate between the plant and animal kingdoms. In its plasmodial stage, the myxomycete of the phaneroplasmodial type is a mass of naked protoplasm containing many nuclei. In this type, the protoplasm flows rhythmically throughout the entire body mass, carrying along the nuclei, vacuoles, granules, and all elements present, inside a narrow gelified periphery.

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We considered that it would be of interest to try to find out how the organelles of the myxomycete plasmodium compared with organelles of other animals and plants, and to see whether we could learn anything about the organization of structures within the protoplasmic mass that might help to explain movement in this organism.

For this study, plasmodia of several species were used, some having typical phaneroplasmodia, similar to the much studied *Physarum polycephalum.* We used *Didymium clavus,* which has a white plasmodium of the phaneroplasmodium type. We also studied *Hemitrichia vesparium,* which has a more delicate plasmodium that is jet black in color, *Stemonitis fusca,* which has a very nearly transparent plasmodium, and *Clastoderma debaryanum,* of the protoplasmodium type. The plasmodium of this species has no veins; it is simply a rounded mass that remains microscopic until the time of fruiting, and fruits to produce only one sporangium per plasmodium. There is no active, reversing flow of protoplasm in this organism.

All of these were prepared by various fixation methods all incorporating osmium tetroxide, sectioned at 40 mμ, and examined in an RCA EMU-3 electron microscope. Phase contrast studies of living and fixed materials were also done.

In the electron micrographs, structures corresponding to most of the organelles described for other species could be identified. For instance, one can see the ground substance, mitochondria, granules of various sizes and having contents that range in density from complete electron opacity to electron transparency. Membrane systems can also be identified, for instance, the rough surfaced endoplasmic reticulum, smooth surfaced reticulum, and Golgi membranes. Ribosomes can be seen as individuals or grouped as polysomes.

One of the most interesting of our findings is the presence in plasmodia of areas of organized structure in the cytoplasm which we have interpreted as fibrils and filaments. Many of the fibrils are of the granular type, and some of them have a substructure which is linearly organized.

Fibrils have been reported by a German worker, Wohlfarth-Bottermann, in droplets and isolated veins of *Physarum polycephalum* (2). Wohlfarth-Bottermann believes that these fibrils insert on such structures as the plasmalemma and vacuolar membrane, and by their contraction cause the movement of the protoplasm that results in the migration of the plasmodium. In our material, we found no evidence of the insertion of these fibrils on any structure that could usefully be moved by them. Occasionally they were seen close to a vacuole.

In some of our plasmodia, there were structures that we have called filaments. These were usually straight, arranged in parallel, and appeared to have an internal area of decreased electron density. They correspond to what others have called "microtubules." Although usually straight, we occasionally did see them variously curved and arched. These structures are very similar in appearance to filaments or microtubules that have been reported from a variety of other cell types. Their function is unknown. We have not found them to insert on any structure, and our interpretation is that they seem more likely to function in conduction of materials through the cytoplasm than in contraction.

By phase contrast we were able to see structures that probably correspond to the fibrils and filaments seen by electron microscopy.

The mitochondria that we saw in all species investigated were of the tubular type, commonly seen in the protozoa. The mitochondrial matrix was much more electron dense than the surrounding cytoplasm. Nicklowitz in 1957 reported in a study of the fine structure of *Badhamia utricularis* that the interior of the tubular cristae opened directly into the cytoplasm so that the contents of the cristae were continuous with the cytoplasm (3). Terada reported a study of *Physarum polyceplalum* in 1962, in which he observed that "it seems that the limiting membrane of the mitochondria has a few pore-like structures, through which the cytoplasmic matrix is connected with the inner matrix of mitochondria" (4). We therefore examined our micrographs carefully to find such evidence in the species which we were studying. We did find configurations that could be interpreted in either of these ways but could not be sure that the appearance was not produced by foldings or irregularities of the surface of the mitochondrion in the particular plane of section.

As is well known, the origin of mitochondria is a controversial question. It has been suggested that they arise *(1)* only by division of pre-existing mitochondria, for which there is good evidence in the ciliate, *Urostyla* (5), *(2)* by the pinching off of parts of the cell membrane, and there is evidence for this in certain malarial parasites (6), *(3)* from small undifferential vesicles in the cytoplasm (7), *(4)* from folds of the nuclear membrane (8), and *(5)* from kinetoplasts (9).

We looked for evidence of this in our material and frequently found mitochondria that seemed in the process of division. We often found what seemed to be mitochondria in stages of breakdown, and many had empty spaces within them.

Mitochondria have been thought to be involved with the formation of yolk in developing oocytes (10). Both light and elec-

tron micrographic studies have been interpreted in this way. Beams (10) found dense bodies within mitochondria of developing oocytes of crayfish, but doubted that they were directly involved with yolk synthesis. In many of our micrographs dense bodies were seen within mitochondria. We also found large dense bodies having somewhat the same appearance as the protein yolk bodies of oocytes. These were particularly numerous in the microscopic plasmodium of *Clastoderma debaryanum.*

Membrane bounded vesicles of a variety of sizes were found in the cytoplasm of all species studied here. The contents of these varied in density and in coarseness of granulation. They were comparable in appearance to what have been described as secretion granules and lysosomes. No centrosome-like structure was ever seen in our micrographs. Golgi membranes were seen; they have the typical appearance of those seen in animal cells.

The plasma membrane of the plasmodium has the usual three layers—two outer dense layers separated by an electron transparent layer. The thickness varied somewhat, but ranges about 30 mμ. A slime network is seen external to the plasmodium.

The membranous organelles of the plasmodial stage of these myxomycetes have thus been found to correspond fairly closely to those found in higher animals and in some protozoa.

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