Fine Structure of the Filamentous Ascomycete *Monosporium apiospermum*

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Introduction

Monosporium apiospermum, the imperfect form of *Allescheria boydii*, is a fungus found in the soil in tropical and subtropical and less often in temperate regions. It has been implicated frequently in infections of the human foot (Madura foot) and lower extremities, and less frequently in nasal and eye infections. Attention was focused on this form as determinations were made of its relationship to an acutely infected, ulcerated human cornea. There existed no previous work on the fine structure of this organism from pure culture. This study was undertaken to elaborate the fine structure of *M. apiospermum* and to form a basis for continuing histochemical studies of "opportunistic" fungi especially those having ophthalmological significance.

Materials and methods

Cultures of *M. apiospermum* obtained from an infected cornea were incubated at 25°C in Sabouraud's liquid medium for 12–24 hours. Mycelial mats were lifted and fixed in 3% glutaraldehyde in 0.01M phosphate buffer and on the succeeding day fixed for 1 hour in 1% osmium tetroxide in phosphate buffer. The fungus was dehydrated in an alcohol series, embedded in Maraglas (Freeman and Spurlock 1962), and sections 100–70 m μ (Peachey 1958) taken for electron microscopy. The sections were stained in lead citrate (Reynolds 1963) and examined on naked grids in Elmiskop 1.

Observations

The fine structure of *M. apiospermum* as studied in this section is described below.

The cell wall. Previous studies have demonstrated chitin as a major constituent of the cell wall of other fungus forms (Blumenthal and Roseman 1957). The cell walls of *M. apiospermum* were positive with the periodic acid-Schiff test for polysaccharides. Their average thickness was ca. 80m μ . The inner aspects of the limiting wall was gently undulating but relatively smooth. The outer aspect of the wall was very irregular having fibrous clumps giving the appearance of sloughing away. The body of the wall consisted of an apparently fibriform meshwork.

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Figs. 1-3. 1, mature mycelium of *M. apiospermum*. Ribosomes are generally dispersed throughout the cytoplasm. The cell membrane rests snugly against the cell wall. N-Nucleus, n-nucleolus, e-endoplasmic reticulum-golgi complex. 2, section of a mature mycelium showing a complete septum in cytoplasmic inclusion bodies. e-endoplasmic reticulum-golgi complex, i-inclusion bodies, s-septum. 3, typical mitochondria in a mature mycelium cross section. m-mitochondrion.

Cell membrane. The highly convoluted cell membrane ca 15 millimicrons in diameter rests against the cell wall except at places where there are observed many invaginations and in some instances membrane enclosed sacs. At many places an overlapping of the cell membrane was accompanied by pores of varying sizes which were occluded only by the electron opaque material dispersed generally between the cell wall and the

cell membrane.

Septa. The apical tips of young mycelia were coenocytic; the mature mycelia were septate. The septa were ca. 70 $m\mu$ in thickness. However, where the septa affixed itself to the main wall, the connections were buttressed considerably.

Reticular system and golgi complex — Grnaules corresponding to the RNA granules previously described (Palade 1958) were generally distributed throughout the cytoplasm but had no association with membranes.

Many instances were observed where the cytoplasm contained dual membrane

complexes. Other cross sectional configurations of such complexes revealed dual membrane associated with clusters of vesicles. The whole considered in three-dimensional aspect represented clearly neither the typical endoplasmic reticulum nor the typical golgi complex. On the contrary, there were characteristics of both E.R. and golgi. Relationships between the E.R. and golgi have been described (Ziegel and Dalton 1962) which would not discount the possibility that primevally these two cellular organelles were indistinguishable in form and complementary in function.

Mitochondria. The mitochondria were little different than those described from other material (Palade 1952); however, no extensively elongated forms were demonstrable. In some of the slightly elongated mitochondria, the cristae ran parallel to the long axis of the body. The dual nature of the mitochondrial membranes and of the cristae was apparent.

Nucleus. The Feulgen positive nucleus averaged 1.5 to 2.1 microns in diameter. It was bound by a dual membrane the inner one being serrate. Intra-nuclear blebs were seen occasionally associated with the inner nuclear membrane. The nuclear matrix consisted of a slightly opaque material interspersed by an interconnecting reticulum. This reticular network contained irregularly spaced electron dense bodies ca. 0.5–1 millimicrons, the significance of which have not been determined. Several nuclei may have been present in a single cell. Nucleoli ca. 150 millimicrons in diameter with an internal structure were observed in most nuclei. The inner nuclear membrane in some instances extended into the nuclear matrix to enclose or communicate with the nucleolus (LaFontaine 1958).

Cytoplasmic inclusion bodies. Many electron dense bodies were dispersed generally throughout the cytoplasm. These bodies were ca. 1 micron in diameter and were seen in one plane to consist of 12–30 subunits 1–2 millimicrons in diameter. The functional significance of these granules is not known although they have been represented previously as lipid or polysaccharide storage granules (Shatkin and Tatum 1959).

Discussion

Recent improvements in fixation and embedding permitted a higher quality preservation of fungus fine structure. There were no observable explosion artefacts and electron staining varied only as the fixation buffer was varied.

There is little information in the literature which would serve as a basis of comparison of the fine structure of *M. apiospermum* with that of filamentous fungus forms. In the absence of this point of departure, at least, at present, attention can be given to similarities to previously described distantly related forms and to the uniqueness of this organizational structure as may relate to its mode of existence.

There has been visualized a functional relationship between well defined golgi complexes and ergastoplasm of some cell types (Ziegel and Dalton 1962).

The absence of a clear distinction between the E.R. and the golgi complex and the relationship of the two to the ribosomes in *M. apiospermum* poses questions on the comparative evolutionary significance of these relationships as opposed to the same organelles in more evolutionarily advanced forms. In cells of advanced forms, the ribosomes may be free in the cytoplasm or associated with membranes as in the protein secreting cells of the pancreas. It has not been established whether the membranes of the golgi and E.R. are present even in the absence of secretory product or whether the surface interface membranes arise as ribosome activity accumulates a lake of secretory product. However, it has been established that the ribosomes of the ergastoplasm play a major role in the synthesis of proteinaceous secretory products of cells.

The ribosomes of *M. apiospermum* are not associated with membranes although their secretory product accumulates in golgi-like membranous cisternae and vesicles near the periphery of the cell. Correlated biochemical and morphological observations support the idea that the golgi system functions in secretory cells as a segregation and concentration center (Caro and Palade 1964), a concept which previously was supported largely by inferential evidence. The golgi complexes of *M. apiospermum*, some showing density comparable to a contained colloid, may well be the storage site of proteolytic product. The placement of these complexes near the periphery of the cell would at least enhance a ready exit through the cell membrane. The production and accumulation of a proteinase within the cell without destruction of the cell has been demonstrated. Lack of cellular destruction may not rest so much on enzyme specificity (Bergmann 1942) as upon the inability of cellular protein to be attacked unless it is slightly denatured through metabolic wear and tear (Oginsky and Umbreit 1959). The fibriform nature of the cell wall in addition to previously described canaliculi would provide mode of exit of proteolytic enzymes to the exterior environment.

Of interest also was the presence within the thallus of other large electron dense bodies which on closer examination were seen to be composed of smaller subunits. Their specific role in the metabolism of the cell or in the production of collagenase has not been determined. It was suggested previously that these were polysaccharide or lipid storage granules; however, existing knowledge does not permit us to discount a possible role of these structures in the production of proteolytic enzymes.

An underlying concern in the study of this form was structural modification which would relate to its invasive ability. The efficiency of *M. apiospermum* in collagen dissolution has been shown repeatedly (Pautler, Roberts and Beamer 1955). From the human eye there has been reported an enhancement of the invasiveness of this and other fungi in the presence of steroids and antibiotics (Zimmerman 1962). Whether the bacterial antibiotics are ineffective in fungus control or whether they enhance the virulence of the fungus is not known. Steroids are believed to act in the dissolution of the ground substance thereby exposing more readily the collagen

bundles to destructive enzymes.

Whereas in this morphological study of *M. apiospermum* several singular characteristics are described which may relate to its role in collagen dissolution it is seen that additional studies are indicated to fully characterize the interrelated role of cellular organelles in elaboration of proteolytic enzymes.

Abstract

A study was made of the fine structure of *M. apiospermum*, the imperfect form of *Allescheria boydii*. The hyphae, either coenocytic or discretely cellular demonstrated those organelles previously described from plant and animal cells. The primitive configuration of the reticular system-golgi complex was noted and its possible role in enzyme storage characterized. In addition, there were described from the cytoplasmic matrix many electron dense bodies composed of repeating subunits. Questions were presented as to the role of these bodies and golgi "secretions" in the proteolytic activity of this fungus form.

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