Wall Structure of a Bitunicate Ascus

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Received December 18, 1970 / February 15, 1971

Summary. The bitunicate ascus develops in two stages prior to ascospore formation: 1) initial growth and expansion of the ascus mother-cell, and 2) deposition of a secondary wall layer, the endotunica, within the outer primary wall layer, the ectotunica. The layers of the bitunicate ascus are composed of microfibrils embedded in an amorphous matrix. The ectotunica and the endotunica differ only in the arrangement of the microfibrils. The primary wall layer is deposited during growth and expansion of the ascus mother-cell; the microfibrils are parallel to the ascus-protoplast surface. The secondary wall layer is deposited after the ascus mother-cell has fully expanded and before ascospore formation; the microfibrils are arranged in a banded pattern. Expansion of the ascus wall during ascospore expulsion is accomplished by a rapid reorientation of the microfibrils of the secondary layer.

Introduction

The unitunicate and bitunicate are two basic ascus types recognized in contemporary mycological literature (Alexopoulos, 1962). Although the construction of the ascus wall is the major criterion for the recognition of these types, the traditional research emphasis in ascus development has been on cytological events leading to ascospore formation.

The unitunicate ascus has a wall consisting of a single layer. An apical thickening occurs in some species in the form of an inner ring. Ascospores may be discharged from the unitunicate ascus by explosion or dissolution of the ascus wall or via an apical opening which may be preceded by an extension of the tip of the ascus.

The bitunicate ascus wall has two layers—an outer termed exotunica or ectotunica, and an inner, the endotunica or endotunica. This type is unique in that the inner wall-layer rapidly extends out past the original boundary of the primary wall preceding spore ejaculation. Chadefaud (1942) described "les asques à nasse apicale bien distincte" which he correlated (1954) with the bitunicate ascus defined by Luttrell (1951). Such an ascus is characterized by 2-4 more or less twisting, longitudinally oriented, tapering bands formed at the apical interior of the ascus. Funk and Shoemaker (1967) suggested that the inner tunica of the bitunicate ascus was a multilayered structure.
The objective of this fine-structure study of the bitunicate ascus is to elucidate the correlation between electron-microscopic detail of the ascus-wall structure with the images seen with the aid of light microscopy.

Materials and Methods

The fungal isolate used in this study was isolated from a "sooty mold" colony collected in Belem (Amazonas, Brazil) during the summer of 1969, and is assigned to *Limacinula theae* Syd. & Butl. Fruit bodies were rolled across the surface of 4% agar to rid them of as much surface contamination as possible. The ascocarps were then teased apart, and the ascospores and fruit-body parts inoculated onto fresh plates of 1/3-strength Fraser's agar (1934). The strain so isolated was maintained on Fraser's agar.

Several fixation procedures were tried on this isolate including use of KMnO₄ and combinations with formaldehyde, picric acid, glutaraldehyde, acroleine and OsO₄. The KMnO₄ fixation failed completely; the other formulas were moderately successful. The most workable fixative proved to be 6% glutaraldehyde, 6% acroleine and 0.5% dimethylsulfoxide (DMSO) in 0.5 M cacodylate buffer, pH 7.2.

The fungus material to be fixed was cut into small discs from the agar with a No. O cork borer and the excess agar pared away. These discs were placed in the fixative within a vacuum chamber at room temperature for 4-8 hr, and kept at 4°C for 12-48 hr. The material was then thoroughly rinsed in 0.5 M cacodylate buffer, pH 7.2, soaked overnight in fresh buffer solution, and further fixed with cacodylate-buffered 2% OsO₄ for 2 hr at room temperature. After this, it was thoroughly washed in distilled water, soaked overnight in 0.5% uranyl acetate at 4°C, and dehydrated in a graded series of ethyl alcohol concentrations up to absolute ethanol. The material was then infiltrated with Spurr's (1969) ERL-4206 low viscosity epoxy resin, using the hard mixture. Sectioning was done manually with a diamond knife on an LKB MT-1 ultramicrotome. Post-section staining was done with Reynolds' (1963) lead citrate for 15 min. The sections were examined with an RCA EMU-3F electron microscope.

The fixation and embedding procedure used is still not ideal for this fungal material. Methacrylate, Araldite and Epikote epoxy resins infiltrated very poorly. With the ERL-4206 it was only possible to cut light to medium gold sections (ca. 900-1000 Å). Thus, the structure of many organelles was not well resolved.

Material for light-microscopic examination was fixed in weak chromacetic acid solution (Johansen, 1940) for 8 hr and washed in distilled water for 8 hr. Staining was done with aceto-carmine and aceto-orcin for 10 min in a 60°C oven after hydrolysis in 1N HCl for 15 min at the same temperature.

Results

Prior to meiosis and ascospore formation, ascus development occurs in two distinct stages: 1. enlargement of the ascogenous cell to full ascus size and formation of a primary wall, 2. deposition of a secondary wall layer. Later there is a rearrangement of the secondary wall components during the ascus elongation which occurs immediately prior to ascospore expulsion.

*The Primary Wall of the Ascus.* The ascogenous cell is subtended by a binucleate cell (Fig. 2). During enlargement of the ascogenous cell to