FINE STRUCTURE OF MYCOTA

4. The Occurrence of the Golgi Dictyosome in the Fungus *Neobulgaria pura* (Fr.) Petrak

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ABSTRACT

Though the dictyosome of the Golgi apparatus appears to be generally distributed in plant and animal cells, it is here described for the first time in the fungi. The present report illustrates, in electron micrographs of thin sections, the characteristic structure of the Golgi dictyosome in a special cell type of a supporting pseudo-tissue (the inner ectal excipulum) of a highly evolved Ascomycete, *Neobulgaria pura* (Fr.) Petrak, a monotypic discomycete. This organelle may secrete the gelatinous matrix filling the cup formed by the inner ectal excipulum. All the other cells in this species appear more typical of fungal cells; i.e., they have no dictyosome and, unlike the cup-forming cells, they show characteristic continuities of the plasma membrane with the perinuclear cisternae. The dictyosome, in those cells in which it appears in this fungus, is formed by a series of vesiculations of the outer component of the nuclear envelope that align to form a stack of sacs. The sacs near the nucleus are flattened (by what appears to be an intermembrane cement) while those near the plasma membrane are more distended. These observations suggest three possibilities: first, fungi may be more closely related to other eukaryotic cells than previously suspected from electron microscopic studies; second, the outer nuclear membrane may have been the primitive predecessor of the dictyosome; and third, the inverse relationship of the occurrence of the nuclear membrane plasma membrane continuities and the dictyosome suggests that the latter may have evolved as a means of removing from the cell the products of reactions occurring on a discontinuous membrane system.

INTRODUCTION

The Golgi dictyosome is most generally characterized by a stack of flattened sacs from the margins of which vesicles sometimes appear to be pinched off. In animal cells where this structure has been most frequently noted it is often involved in secretory processes (35, 42-45) and it very likely has a similar function in some higher plant cells (27). While a similar appearing structure has been described in a moss (19), in some algae (17, 18, 20-22, 36, 37), and protozoa (5, 10-14, 34, 38), its function here is less clear than in the aforementioned organisms. Generally speaking, the distribution of the dictyosome corresponds closely to the distribution of organized nuclei and it is considered to be an ubiquitous organelle of advanced cell types. In a survey of nearly 50 genera of the Eumycota we have noted that while nuclei are consistently present they differ markedly from the nuclei of other major groups of nucleated cells. The limiting cisternae of fungal nuclei are often directly continuous with the plasma membrane (9, 23, 29). Reports of optical (1-4, 40, 41) and electron microscopic studies (7, 8, 15, 16, 46) suggest that the nuclear envelope commonly does not break down and reform during somatic karyokinesis, and that
only rarely are mitotic figures to be observed (24-26). At first glance the fungi, because of the simplicity of their membrane relationships, their lack of a Golgi complex, and their mode of cell division, would seem to represent an intermediate stage in the secondary evolution of cells. However, like other cells above the level of the Schizomycota, they possess nuclei, mitochondria, and well developed cilia in some Phycomycetes (6). Two previous reports (6, 9) tentatively identified vesicle aggregations as belonging to the Golgi complex; the present report, however, presents evidence for the presence of a Golgi system on the less controversial identification of the Golgi dictyosome. This organelle has been found to occur in an unusual fungous cell type in which the nuclear membrane is discontinuous and appears to break down during cell division in a manner comparable to that occurring in mitosis. This report also provides direct evidence for the origin of the dictysome from the outer membrane of the nuclear envelope.

MATERIALS AND METHODS

Substrate

Apothecia (fruiting bodies) of the discomycete, Neobulgaria pura (Fr.) Petrak, were collected October, 1960, in the northern Adirondack Mountains, New York State.

Method

The apothecia were prefixed in 1 per cent unbuffered K\textsubscript{2}MnO\textsubscript{4} for 9 minutes. (A discussion of this method has been presented in the first paper in this series (28).) Subsequently, they were fixed in 2 per cent OsO\textsubscript{4} for 3 hours at 4°C, washed in buffer, dehydrated in an ethanol series, and embedded in a mixture of butyl and methyl methacrylates (90:10). Sections were cut on a Porter-Blum microtome with a Fernández-Morán diamond knife and subsequently examined in a Siemens Elmiskop I.

OBSERVATIONS

The gross morphology of the fruiting body (apothecium) of Neobulgaria pura is shown semi-diagramatically in Fig. 1. In general, the apothecium is a bowl-shaped structure filled with a mixture of gel and diffuse hyphae (the medullary excipulum). At the upper surface of this mixture the hyphae become concentrated into the relatively dense subhymenial layer. From the hyphae of this layer arises a stratum of vertically oriented hyphae, the hymenium, composed of the spore-bearing asci and inter-ascal, sterile paraphyses. The uncomplicated micromorphology of the cells of these mycetec layers (Fig. 10) is typical for fungal cells when compared with observations made on over 50 genera of fungi (28-33). The apothecium is given its shape by the two-layered ectal excipulum. The outer layer is a gel-hyphae mixture comparable to the medullary excipulum in both composition and micromorphology (Fig. 10). However, the inner layer is composed of thick-walled hyphae (textura prismatic\textit{a}) laterally compacted into a firm pseudotissue. It is this inner layer of the ectal excipulum that gives the apothecia their shape and support.

A typical representation of cells from the inner ectal excipulum (Ex) is shown in Fig. 2. These cells display a number of unusual characteristics: 1) the prominent nuclei (N) are more homogeneously light in appearance than the nuclei of most fungal cells; 2) the endoplasmic reticulum (ER) in its most common appearance here is composed of disarticulated vesicular elements that seem to arise from the outer nuclear membrane; 3) the endoplasmic reticulum does not form continuities between the nuclear and plasma membranes as is common in fungal cells and typical in N. pura in cells not of the inner ectal excipulum (Fig. 10).

The most singular feature of the cells of the inner ectal excipulum is the presence of stacks of flattened vesicles (D) of the same appearance as the Golgi dictyosome (Figs. 2 to 8). The nuclear membrane (Figs. 2 to 5, 7, 8) shows some apparent activity adjacent to the dictyosome. In Figs. 5 and 7 the outer nuclear membrane appears to be continuous with the adjacent dictyosome. It would seem, therefore, that the dictyosome does arise from the nuclear membrane in these cells. The flattened sacs proximal to the nucleus (Fig. 6) are compressed by a structured cementing material within them. The peripheral sacs, however, are distended. This gives the impression of a dynamic process from the nuclear membrane to the plasma membrane. Since K\textsubscript{2}MnO\textsubscript{4} fixation destroys ribosomes it is not possible to determine in this preparation if the dictysomes shown here are agranular, as is characteristic of other dictysomes. However, the membranes are so compacted on their adicisternal surfaces that there does not appear to be room between them for any significant number of granules of the diameter of 150 Å.
On occasion (Fig. 9) the nucleus is only partially delimited by cisternae, and these are represented by only a few isolated vesicles surrounding the nuclear phase. This configuration may represent a reformation of the nuclear membrane following division (39). A variety of other inclusions are found in the cytoplasm of the inner ectal excipular cells. These include: 1) a large single membrane-limited body (a) containing a light granular material (Fig. 3); 2) lomasomes (L), structures frequently found in other fungal cells (30) (Figs. 2 to 4, 8, 9); 3) dense globose granules (b) (Figs. 4 to 6) (one of these (Fig. 3) is found within an annulus of the nuclear membrane); and 4) inclusions of low electron opacity (with dense centers) (Figs. 2 to 8).

**DISCUSSION**

The occurrence of the Golgi apparatus was classically considered to be limited to animal cells. Electron microscopic studies, however, have now generally extended the existence of the dictyosome of the Golgi apparatus, its hallmark, to the higher plants, mosses, algae (except Cyanophyta), and protozoa. This establishment of the dictyosome as an ubiquitous organelle in higher cell types would seem to greatly enhance the probability that the structure which is described above is indeed a dictyosome and commonly derived with the dictysomes of other higher cell types. It would, nevertheless, be tenuous on the basis of the above report to express a conviction that the described structure is an authentic dictyosome because it is described in only one cell type of one genus (a highly evolved Ascomycete, in fact). However, while this discussion was in its final draft, a similar

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**Figure 1**

Representation of the fruiting body (apathecium) of the discomycete *Neobulgaria pura* showing the nature and relationship of the structures discussed in the text.¹

¹ We wish to express our sincere appreciation to Professor R. P. Korf for his assistance in the preparation of this drawing, and to Mr. F. C. Reed for his execution of the drawing.
observation of a characteristic dictyosome in an
undescribed monotypic flagellated Phycomycete has
been made and will be subsequently studied. This,
then, provides one example from the carpomy-
cetes (Ascomycetes, Basidiomycetes, and Deu-
teromycetes) and one from the Phycomycetes.
The inclusion of the dictyosome in the Eumycota
is significant in that fungi would seem to be as
phylogenetically distinct from plants as plants are
from animals and protozoa. This discovery
would then establish the occurrence of the dictyo-
some in all major taxa of nucleated cells (except
perhaps the Myxomycota).

The dictyosome might well come to be regarded
as an organelle which was quite primitively de-
uced from internal membrane systems. While its
modern function seems most often to be linked to
secretion, the understanding of its primitive
function may depend on further phylogenetic
studies at the electron microscopic level. A clue
from the present report might deserve further
study. That is the observation that the Golgi-

1 Professor R. Emerson's Costa Rican collection,
number 78.

BIBLIOGRAPHY
1. BAKERSPIGEL, A., The structure and manner of
division of the nuclei in the vegetative my-
celium of the Basidiomycete Schizophyllum

KEY TO LABELING OF FIGURES
a, large vacuolar body
b, dense bodies
c, smaller vacuolar bodies
D, dictyosome
ee, envelope elements
ER, endoplasmic reticulum
Ex, inner ectal excipulum

HW, hyphal wall
L, lomasomes
M, mitochondria
N, nucleus
NE, nuclear envelope
p, nuclear pores

FIGURE 2
Longitudinal section through a pair of cells from the inner ectal excipulum. Two
dictyosomes (D) adjacent to the outer components of the two nuclear membranes
are seen. Vacuolar structures (c), lomasomes (L), nuclear pores (p), and a vesicular
endoplasmic reticulum (ER) are evident. (Ex) indicates the region of the cell walls
of the inner ectal excipulum. X 40,000.

FIGURE 3
Another pair of cells of the inner ectal excipulum showing two other types of inclusions
(a and b), a spherical mitochondrion (M) with radially oriented tubular cristae, and
another dictyosome (D) adjacent to the nucleus (N). Note the symmetrical pair of
diverticula on the outer component of the nuclear envelope adjacent to the dictyosome.
X 50,000.


17. MANTON, I., Observations with the electron microscope on the internal structure of the zoospore of a brown alga, J. Exp. Bot., 1957, 8, 294.


19. MANTON, I., On a reticular derivative from Golgi bodies in the meristem of Anthoceros, J. Biophys. and Biochem. Cytol., 1960, 8, 221.


**Figure 4**

Another cell, similar to those in Figs. 2 and 3, which shows a further example of symmetrical diverticula on the outer component of the perinuclear cisterna adjacent to the dictyosome (D). × 50,000.

**Figure 5**

In this micrograph the region of the dictyosome proximal to the nucleus appears to be in continuity with the outer nuclear membrane. × 50,000.
FIGURE 6
A dictyosome showing compact lamellae adjacent to the nucleus and distended ones adjacent to the plasma membrane. The innermost of these flattened cisternae shows fairly regularly spaced densities between its apposed membranes. \( \times 100,000 \).

FIGURE 7
A diverticulum of the outer nuclear membrane (arrow) appears to align in the plane of the cisternae of the dictyosome. \( \times 100,000 \).

FIGURE 8
Symmetrical diverticula of the outer nuclear membrane appear opposite the dictyosome (arrows) and seem to be related to vesicular elements of the endoplasmic reticulum. \( \times 100,000 \).
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Note added in proof: Since the submission of this paper several complementary observations have come to our attention: (1) The Golgi complex has now been observed in three additional Phycomyete genera. In personal communications it has been noted to occur in Peronospora and Albugo by C. C. Bowen and in Pythium by Lilian Hawker. (E. C. Cantino, however, states that he has failed to find it in his studies of Blastocladiella.) (2) Zeigel and Dalton (J. Cell Biol., 1962, 15, 45) in a recent paper on the micromorphology of the Golgi systems of several animal tissues associated with the secretion of various kinds of proteins offer a number of micrographs to support the hypothesis that blebs from the nuclear envelope may be "one source of the small variety of Golgi vesicles." A similar relationship of the perinuclear cisterna to the Golgi dictysome also has been previously noted by McAlear (unpublished) in the sixth abdominal ganglion of the crayfish and other micrographs of the material shown in the present contribution have appeared previously in an abstract (Moore and McAlear, Electron Microscopy, 1962, 2, UU-7, New York, Academic Press).