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The Structure of *Saccharomyces carlsbergensis* and *S. cerevisiae* as Determined by Ultra-thin Sectioning Methods and Electron Microscopy

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SUMMARY: Cells of *Saccharomyces carlsbergensis* and *S. cerevisiae* were fixed with osmic acid, embedded in plastic, sectioned at one-twentieth of a micron, and then observed in the electron microscope. The pictures obtained gave confirmation of the double membrane nature of the yeast cell wall reported by earlier workers such as Northcote & Horne (1952), and gave some indication of the presence of additional very thin membranes in the cell wall. It was suggested that the two cell wall membranes might be the two osmotic barriers observed to be present in the yeast cell wall by Conway & Downey (1950). No structure was seen which could be identified as the cytoplasmic membrane.

Guilliermond (1920) wrote that at least two investigators, H. Will in 1896 and O. Casagrandi in 1897, had reported that yeast cell walls consisted of two distinct and separate membranes. These early workers reported that the two membranes were seen in older cultures after treating the cells with hydrochloric acid, and that osmic acid was especially good for making the membranes visible. Guilliermond also wrote, and it is common knowledge, that the yeast cell wall develops from a thin membrane into a rather thick membrane as the cell ages. However, the evidence for the double membraned cell wall seems not to have been accepted by most writers of text-books since they generally have failed to mention this point. Impressive evidence for the double cell-wall membranes was recently presented by Northcote & Horne (1952). They obtained preparations of the yeast cell membrane, free from cytoplasm and whole cells, by mechanically rupturing the cells and following this by differential centrifugation. They state that '...when the preparations were treated with 2N-HCl...it could be seen that the cell envelope consisted of at least two membranes'. They also presented an electron microscope picture clearly showing these two membranes in a cell wall fragment of *Saccharomyces cerevisiae*. The demonstration of two membranes in the yeast cell wall is of importance since they might be expected to play an important role in the behaviour of the cell wall as an osmotic barrier. Conway & Downey (1950), also working with *S. cerevisiae*, reported the presence of two osmotic barriers in the cell wall. The outer barrier was reported to be permeable by acetic, propionic, succinic, and glyceric acids, but not by inulin or peptone. The inner barrier was reported as being permeable by acetic and propionic acids, but not by succinic or glyceric acids. The area into which the succinic and glyceric acids penetrated was shown to be the outer 10-11 % of the total cell volume, and was assumed to represent the cell-wall area.

The present paper presents the results of the application of thin sectioning techniques in an attempt to study the structure of the yeast cell, and in particular the structure of the yeast cell wall.

METHODS

Cultures of *Saccharomyces carlsbergensis* and *S. cerevisiae* were grown for 72 hr. in glucose yeast extract broth at 30° on a shaking machine. The cells were then removed by centrifugation and washed twice with distilled water. The fixing and plastic-embedding procedures were those described for bacteria by Chapman & Hillier (1953) except that in some instances the osmic acid fixation was omitted. Sectioning was accomplished with a Spencer microtome adapted for thin sectioning by the method of Pease & Baker (1948). Razor blades were sharpened to an angle of 52° and used for the cutting edge. The sections were cut with the microtome set for one-twentieth of a micron and then placed directly on electron microscope grids and observed in a model 50 R.C.A. electron microscope.

RESULTS

Plates 1 and 2, and Pl. 3, figs. 3 and 4, show osmic acid-fixed and sectioned cells of *Saccharomyces carlsbergensis*. In all cases where the protoplasm shrank away from the cell wall, at least two lines are shown in the cell-wall area. In the areas where the protoplasm remained in contact with the cell wall, the inner line was difficult to detect, but could be seen in some pictures. The usual picture obtained by sectioning is represented by Pl. 2, fig. 2. The two areas seen in the cell wall are consistent with the double membrane cell-wall structure demonstrated for *S. cerevisiae* by Northcote & Horne (1952). If the limits of the two cell-wall membranes shown in Pl. 2, fig. 2 are taken, the cell-wall width measures 0.16 μ . Northcote & Horne believed that possibly more than two membranes were present, since they reported seeing several thin layers peeling away at the site of the bud scars following the treatment of their cell-wall preparations with HCl. A similar peeling is indicated in our pictures by the arrows at the site of the new bud shown in Pl. 3, fig. 6, which is just beginning to push through the old cell wall of the mother cell. Additional evidence for several layers in the cell wall is indicated by the laminations in the cell wall indicated by the arrows in Pl. 1, fig. 1 and Pl. 3, fig. 3. The outer and inner membranes of the cell wall seem to consist of closely packed thin layers of material, whereas the less dense central area seems to consist of less well-developed layers, not so closely packed together. The bottom part of the cell shown in Pl. 3, fig. 4 (see arrow) is interesting since it shows a cut directly through a bud scar. Note that the double cell-wall membrane persists through the bud scar.

A comparison of the cell-wall development shown by the mother and daughter cells in Pl. 3, fig. 6, is of interest. Henrici (1947) stated that the cell wall of young cells is thin and increases in thickness with age. This is clearly indicated in Pl. 3, fig. 6, since the mother cell has a rather well-

developed cell wall, while the cell wall of the daughter cell is comparatively thin. This then is direct proof that the cell wall does increase in thickness with age, and the thick cell wall shown in Pl. 4, fig. 7 represents the cell wall of an older cell (or 'dauerzellen' of the older literature).

Plate 3, figs. 5 and 6, are sections through osmic acid-fixed cells of *Saccharomyces cerevisiae*. Note the presence of the inner cell-wall membrane shown in Pl. 3, fig. 5. The double cell-wall membranes are faintly shown in Pl. 3, fig. 6, but *S. cerevisiae* did not give as clear results as did *S. carlsbergensis*. Pl. 4, figs. 8–10, show sections of *S. carlsbergensis* in which osmic acid fixation was omitted. Notice that the cell wall is not nearly as well delineated as in the sections where osmic acid was used. In fact, little of the double membrane nature of the cell wall is in evidence. It is also obvious that the protoplasm was not nearly as shrunken nor as opaque to the electron beam as when osmic acid was used as a fixative. This resulted in the demonstration of some interesting electron-opaque bodies in the cell vacuole. They appeared either large, single, or paired, as in Pl. 4, figs. 9 and 10; or small paired, or in chains, as in Pl. 4, fig. 8. Attempts were made to identify these bodies as volutin by extraction from the intact cell by appropriate methods before embedding and sectioning, but these procedures resulted in cells which were valueless for observation after sectioning. These bodies are identical in appearance with those termed mitochondria by Sarachek & Townsend (1953), but undoubtedly might also be the structures often referred to as volutin, lipoidal granules, metachromatic granules, or even possibly the chromosomes of Subramanian & Ranganathan (1945).

The sectioned cells shown here are all from 72 hr. cultures; when 24 hr. cultures were used the resulting preparations gave such poor quality sections that little could be determined in regard to cell structures.

DISCUSSION

The pictures shown in this paper appear to confirm earlier reports of the presence of at least two membranes in the yeast cell wall. However, it must be admitted that some problems of interpretation of the pictures exist. Some could argue that the outer line shown in the photographs represents adsorbed dirt, and the inner line represents adhering protoplasm. We do not believe this to be the case since these lines did not appear in the cell walls of similarly treated cells not fixed with osmic acid (compare Pl. 2, fig. 2 and Pl. 4, fig. 8), and all of the cells were washed several times before the embedding steps were begun. In addition, Guilliermond (1920) reported Will's statement that the double membranes in the cell wall were made more visible by treatment with osmic acid. One would expect that this would hold also for electron microscopy since osmic acid is known to make materials electron-opaque. Thus the osmic acid could act as a stain for these membranes. Close inspection of the enlarged cell wall shown in Pl. 3, figs. 3 and 5 (and to a lesser extent in Pl. 1, fig. 1), shows wavy structures constituting the inner membrane which would be difficult to explain on the basis of adhering protoplasm.

It seems highly probable that these pictures give cytological confirmation of the two separate permeability barriers reported for the yeast cell wall by Conway & Downey (1950) who used physical chemical methods. If one calculates the cell volume included by the two cell-wall membranes shown in Pl. 1, fig. 1, it turns out to be *c.* 10 % of the total cell volume. This is in agreement with Conway & Downey who found by their calculations that the yeast cell wall was bounded by two different permeability barriers and that the cell wall occupied from 10 to 11 % of the total cell volume. It logically could be concluded that the outer line shown in our pictures represents the outer permeability barrier (impermeable by inulin and peptone, but permeable by succinic, glyceric, acetic and propionic acids), and the inner line in our pictures represents the inner permeability barrier (impermeable by succinic and glyceric acids, but permeable by acetic and propionic acids) reported by Conway & Downey.

The question can arise about the possibility of the inner line representing the cytoplasmic membrane of the yeast cell. Robinow & Murray (1953) demonstrated the cytoplasmic membrane of bacteria and yeast by a staining method and observation with the light microscope. In their pictures the cytoplasmic membrane adhered to the protoplasm and shrank away from the cell wall on plasmolysis. It is obvious that the inner cell-wall membrane demonstrated by our pictures is sufficiently rigid to remain as an outer structure after the protoplasm has retracted. For this reason we believe it to be part of the cell wall and not the cytoplasmic membrane. It is not surprising that a cytoplasmic membrane was not demonstrated in our sections of yeast since Chapman & Hillier (1953) also failed to demonstrate it in their sections of bacteria. It is possible either that the membrane was too small for resolution, that it lacked electron-scattering powers, or that it was destroyed by the treatments involved in embedding and sectioning.

The electron opaque structures shown in Pl. 4, figs. 8–10, are interesting. These have the same appearance as the ‘mitochondria’ reported for yeast cells by Sarachek & Townsend (1953). These authors reported that the ‘mitochondria’ were small in actively metabolizing yeast, and that they coalesced into larger particles as metabolism slowed. This would mean that the smallness of the particles in Pl. 4, fig. 8, would be due to a high metabolic rate for this cell at the time of fixation, while the larger particles shown in Pl. 4, figs. 9 and 10 would be due to their presence in an older cell with a lower metabolic rate. Sarachek & Townsend reported that the large forms originated by coalescence of the smaller bodies, and they would interpret the paired bodies shown in Pl. 4, fig. 10, as being in the act of coalescence. However, it is not certain that these structures should be called mitochondria, and it would be just as well to call them volutin, lipoidal granules, or metachromatic granules. It is also possible that these are the tennis-ball-shaped chromosomes of some authors (Subramanian & Ranganathan, 1945). We have not yet been able to identify the nature of these particles, but we can report that they are highly volatile under an intense electron beam since they often disappeared as they were being observed in the electron microscope.

J. W. BARTHOLOMEW & R. LEVIN—YEAST STRUCTURE AS SHOWN BY ULTRA-THIN SECTIONING.
PLATE I

(Facing p. 476)

J. W. BARTHOLOMEW & R. LEVIN—YEAST STRUCTURE AS SHOWN BY ULTRA-THIN SECTIONING.
PLATE 2

J. W. BARTHOLOMEW & R. LEVIN—YEAST STRUCTURE AS SHOWN BY ULTRA-THIN SECTIONING.
PLATE 3

J. W. BARTHOLOMEW & R. LEVIN—YEAST STRUCTURE AS SHOWN BY ULTRA-THIN SECTIONING.
PLATE 4

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. *S. carlsbergensis*, osmic acid fixed, embedded in plastic, sectioned at one-twentieth of a micron. Showing the double membrane nature of the cell wall.

PLATE 2

Fig. 2. Same as fig. 1.

PLATE 3

Fig. 3. An enlarged portion of fig. 2.

Fig. 4. *S. carlsbergensis*, same treatment as figs. 1 and 2. Showing a section through a bud scar, and the double membrane nature of the cell wall.

Fig. 5. *S. cerevisiae*, osmic acid fixed, embedded in plastic, sectioned at one-twentieth of a micron. Showing cell-wall structure. Note the fibrous nature of the inner cell-wall membrane.

Fig. 6. Same as fig. 5. Showing a section through a young bud, and a section through two newly forming buds.

PLATE 4

Fig. 7. *S. carlsbergensis*, same as figs. 1 and 2. Showing the thick cell wall of an old cell.

Figs. 8-10. *S. carlsbergensis*, osmic acid-fixation step omitted, otherwise the same as fig. 1. Showing internal electron-opaque granules which were masked when osmic acid was used as a fixative. These granules have been variously termed volutin, mitochondria, chromosomes and lipoidal granules.

The length of the scale in each figure is 1μ .

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