The Formation of Buds in Yeast

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SUMMARY

Vesicles accumulate at the site of bud formation in the mother cell and are also found in the growing bud during the growth of its wall. The bud is in direct communication with the mother cell until maturity when a septum which is formed across the cytoplasm grows inwards from the wall and is subsequently thickened on both sides. During the thickening process vesicles are present at both surfaces of the cross-wall which finally has two layers one of which becomes continuous with the bud cell wall. It is suggested that the vesicles carry material both into the growing bud wall and into the septum.

INTRODUCTION

The relationship between cytoplasmic organization and wall formation in yeast cells is a problem of long-standing interest and the search for organelles which are involved in the budding process has led several authors to examine thin sections of cells with the electron microscope. But the fixatives generally used—osmium tetroxide and potassium permanganate—are of limited value as they contain heavy metals and are oxidizing reagents so that they distort important structures.

To overcome fixation problems Mundkur (1960*a, b* and 1961) applied a freeze-drying technique and Moor & Mühlethaler (1963) and Moor (1967) adapted the freeze-etching technique of Steere (1957) to the study of yeast. This latter technique permits the stabilization of both prolonged and transitory stages of structures that are not preserved when ordinary chemical fixation is used but the replicas obtained are difficult to interpret and therefore it is desirable to correlate the image with those obtained by other techniques.

Aldehyde fixatives, first introduced by Sabatini, Bensch & Barnett (1963) have greatly improved our knowledge of the fine structure of animal and plant cells. Fixation occurs by cross-linkages between various chemical groups of the polymers so that there is only slight distortion of the cellular structure.

Robinow & Marak (1966) and Schmitter & Barker (1967) fixed yeast with glutaraldehyde before or after removal of the cell wall with snail gut enzyme in an attempt to obtain better intracellular resolution.

In this study the ultrastructural morphology of glutaraldehyde fixed *Saccharomyces cerevisiae* has been re-examined with special reference to the budding process.

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METHODS

Organism. A strain of *Saccharomyces cerevisiae* isolated from commercial pressed baker's yeast (Distillers Co. Ltd.) was used. This strain was maintained on Winge medium (yeast extract, 3 g.; glucose, 20 g.; water, 1 l.) in 2% agar slopes at 4°; it was cultured in Winge medium agitated in a reciprocal shaker at 28°. The yeasts were harvested at the beginning of the logarithmic phase of growth and washed twice in ice-cold distilled water.

Electron microscopy. The yeast cells were fixed in a glutaraldehyde fixative (4% glutaraldehyde in 0.02 M-phosphate buffer pH 6.8, with an added salt mixture, (Zetterquist, 1956)) at 21° for 1 hr, and washed four times with the same buffer over a period of several hours. Post-fixation was done in 1% osmium tetroxide solution in veronal buffer (0.05 M, pH 7.0) for 1 hr. The pellet was embedded in 0.75% agar blocks, dehydrated with an ascending ethanol series and embedded in araldite resin.

Sections were cut with glass knives on a Sorvall Porter-Blum ultramicrotome MT 2. Gold sections were collected on carbon coated copper grids and stained with uranyl acetate (saturated solution in 50% ethanol) at 60° for 15 min. followed by lead citrate (0.09% lead; Reynolds, 1963) for 1 min. The sections were examined by a GEC-AEI, EM6B electron microscope at 80 kV.

RESULTS

The use of glutaraldehyde as a fixative resulted in satisfactory preservation of the organelles known to be present in the yeast cell and showed an unusually complex organization of its membrane system.

The plasmalemma had small invaginations into several regions of the cytoplasm; some of these invaginations were almost circular in section but others showed much greater structural complexity (Pl. 1, fig. 1, 2, 4); they resembled packages of more or less concentric rings or aggregates of membranes (Pl. 1, fig. 1, 4). They were sometimes seen in close contact with the endoplasmic reticulum (Pl. 1, fig. 1).

The cell wall appeared on the electron micrographs as a layer of low electron density with the plasmalemma in close contact with it. The initial stage in the budding process became apparent at sites just under the plasmalemma where an accumulation of vesicles occurred (Pl. 2, fig. 5, 6, 7). The cell wall in this region bulged out and vesicles accumulated in the underlying cytoplasm (Pl. 2, fig. 5). The cell wall of the growing bud increased in area but not in thickness (Pl. 2, fig. 6, 7) and organelles other than vesicles and endoplasmic reticulum accumulated in the bud. Before the bud reached maturity a cross-wall began to be laid down at the junction between the bud and the mother cell (Pl. 1, fig. 3, Pl. 3, fig. 8, 9, 10, 11). This cross-wall which was first seen as a ring between the cell wall and the plasmalemma (Pl. 3, fig. 8), grew centripetally in a similar way to that described for bacteria and other fungi (Pl. 1, fig. 3; Pl. 3, fig. 9). During the formation of this cross-septum vesicles were seen in the cytoplasm alongside it both in the mother and in the bud cell (Pl. 1, fig. 3; Pl. 3, fig. 10, 11; Pl. 4, fig. 12). The contents of the vesicles had the same appearance as the material which made up the septum. The vesicles were similar in appearance to those involved in the formation of the bud cell wall. The cross-wall had a sinuous shape and was displaced towards the mother cell (Pl. 1, fig. 3; Pl. 3, fig. 9, 10). When the separation between the two cells was completed by the formation of a thin septum, this was

thickened to produce a double-layered cross-wall (Pl. 4, fig. 13, 14). The septum was lined on each side by plasmalemma which was continuous with that of the bud or of the mother cell. A gap appeared between the two layers but the cells remained attached around the ring formed on the mother cell wall (Pl. 4, fig. 13). The septum on the bud cell side became continuous with the wall but separate layers were clearly distinguished between the septum and the mother cell wall (Pl. 4, fig. 13, 14).

The birth scar had only one layer while the bud scar on the mother cell had at least three, the outermost layers being part of the mother cell wall, and the inner layer, part of the cross-wall (Pl. 4, fig. 15).

Fig. 1. Diagrams to show the sequence of bud and septum formation. The bud cell is the upper cell and the mother cell is the lower cell.

(a) and (b). Vesicles accumulate under the wall during the initial stages of bud formation. (Compare Pl. 2, fig. 5, 6 and 7.)

(c) Nuclear material, mitochondria and endoplasmic reticulum are passed into developing bud.

(d-g). Formation of the septum across the connexion between the bud and mother cell. The septum is formed by the incorporation of material passed to it in vesicles from both sides. It grows from the wall of the cell inwards. (Compare Pl. 1, fig. 3; Pl. 3, fig. 8, 9 and 10.)

(h) The septum is thickened and forms two layers. (Compare Pl. 3, fig. 11; Pl. 4, fig. 12.)

(i) A gap appears between the layers of the septum. One layer is continuous with the wall of the bud but the other is distinct from the wall of the mother cell. (Compare Pl. 4, fig. 13 and 14.)

(j) The bud is separated from the mother cell. (Compare Pl. 4, fig. 15.)

DISCUSSION

The presence of invaginations of the plasmalemma into the cytoplasm of yeast cells has been clearly demonstrated by freeze etch studies (Moor & Mühlethaler, 1963; Northcote, 1968). The fixation and staining methods used in this work have enabled the membranes to be investigated and the complexity of some of the invaginations can be seen to resemble the mesosomes of Gram-positive bacteria (Ryter, 1968). When the cell walls of bacteria are digested away by lysosyme the protoplasts obtained are spherical and the mesosome is extruded from it in the form of a beaded appendage (Fitz-James, 1966; Ryter, 1968). The blebs which are formed at the surface of the protoplasts of yeast (Svihla & Schlenk, 1965) may have a similar origin.

The fine structure of the yeast cell and the budding process has been studied in thin sections by several workers; however a complete sequence of events has been impossible to describe mainly because of the difficulties of fixation, embedding and staining. By the use of glutaraldehyde as a fixative we have been able to demonstrate the initial stages of bud formation and the subsequent formation of a septum between the mother and the bud cells (Fig. 1*a-j*). The formation of the septum in yeast is seen to be similar to that described for some other fungi and Gram-positive bacteria (Glauert, 1962; Hawker, 1965; Ryter, 1968).

During the initial stages of its formation the bud is extended by new cell wall material that is probably carried to the site in the vesicles which accumulate at the bud region and the material is passed across the plasmalemma by reverse pinocytosis. It has been shown by the freeze etch technique that synthesis of some of the cell wall substance probably takes place from small organized particles at the outer plasmalemma surface (Moor & Mühlethaler, 1963). Thus the cell wall is formed partly off site in vesicles which are transported to the wall and partly at the cell surface from synthetic particles (Northcote, 1968) in a way similar to that indicated for the formation of the cell walls of higher plants (Northcote & Lewis, 1968) and fungi (McClure, Park & Robinson, 1968). The vesicles seen in the sections are equivalent to the 'proteaseparticles' described by Moor (1967) from a freeze etch study, but he suggested that the vesicles carried enzymes for dissolving and weakening the wall at the point of bud formation. The origin of the vesicles is not clear but it is possible that they are derived from the endoplasmic reticulum system (Moor, 1967; Marchant & Smith, 1967).

As the wall grows out of the parent cell nuclear material, mitochondria, endoplasmic reticulum, ribosomes and other cytoplasmic inclusions are passed into the bud region. The bud is finally cut off from the parent cell by a septum which grows inwards from the wall across the intercommunicating region as a thin sinuous strand and is then thickened by the deposition of material from both sides. The thin septum is not seen very frequently in sections containing some hundreds of yeast prepared from an actively growing culture and it is likely therefore that the growth of the septum and its subsequent thickening is quite a rapid process.

During formation of the septum and its thickening, material is again probably deposited from vesicles which can be seen to accumulate at this region at both sides from the mother and from the bud cell. Although the septum increases in thickness its diameter remains fairly constant because of its attachment with the mother cell wall and during the latter stages of its formation the continuity of the material of the

septum on the bud cell side with the wall of the bud can be seen, but such a direct continuity is not clear on the mother cell side. It may be that as the bud grows a tension is developed at the junction which results in the breakage of the connexion and a release of the bud which then rounds off its wall at the birth scar. But the distinctive layered appearance of the septum at the bud scar of the mother cell remains.

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EXPLANATION OF PLATES

Fig. 1-15. Pictures of sections of yeast cells taken with an electron microscope. B, bud cell; M, mother cell; e, endoplasmic reticulum.

PLATE 1

Fig. 1. An invagination of the plasmalemma into the cytoplasm can be seen. The invagination is circular in section and is closely associated with profiles of the endoplasmic reticulum. $\times 87,000$.

Fig. 2. Simple invaginations of the plasmalemma. These are close to profiles of the endoplasmic reticulum. $\times 75,000$.

Fig. 3. Intermediate stage in the growth of the septum across the connexion, between the mother and bud cells. The septum is not continuous at its centre although it is well developed at its outer edge. $\times 19,000$.

Fig. 4. A circular profile of a complex invagination of the plasmalemma into the cytoplasm of the cell is shown. $\times 70,000$.

PLATE 2

Fig. 5. A region in the mother cell at the initial stage of bud formation. Vesicles can be seen to have accumulated in the cytoplasm of the cell just under the plasmalemma. $\times 67,000$.

Fig. 6, 7. Initial stages in the growth of the bud. The cell wall of the bud has extended in area and vesicles can be seen to have accumulated in the cytoplasm in this region of the cell. Fig. 6 $\times 39,000$. Fig. 7. $\times 52,000$.

PLATE 3

Fig. 8. Initial stage in the formation of the septum between the mother and mature bud cell. A transverse section of a circular ridge formed on the wall can be seen. $\times 39,000$.

Fig. 9. A thin septum which extends completely across the connexion between the bud and mother cell can be seen. It is lined on both sides by plasmalemma. $\times 54,000$.

Fig. 10 and 11. Stages in the thickening of the septum. Vesicles can be seen on either side in the mother and bud cells. Fig. 10 $\times 30,000$. Fig. 11 $\times 39,000$.

PLATE 4

Fig. 12. A stage in the thickening of the septum. It is possible that the appearance of the septum on the mother cell side could be caused by the deposition of material from a vesicle across the plasmalemma. $\times 75,000$.

Fig. 13 and 14. Stages in the final thickening and development of the septum. Two layers in the septum are visible. The layer on the bud cell side is continuous with the cell wall of bud. The layer on the mother cell side is distinct from the cell wall. Fig. 13. $\times 50,000$.

Fig. 14. $\times 39,000$.

Fig. 15. Bud scar on the mother cell. $\times 25,000$.