MITOSIS IN HETEROBASIDIOMYCETOUS YEASTS

1. LEUCOSPORIDIUM SCOTTII (CANDIDA SCOTTII)

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SUMMARY

*Leucosporidium scottii*, for many years considered to be an asporogenous yeast in the genus *Candida*, is now known to have a heterobasidiomycetous life-cycle. Although morphologically similar to ascomycetous yeasts such as *Saccharomyces cerevisiae*, budding cells of *L. scottii* have a strikingly different type of mitosis. In *L. scottii*, mitosis is not intranuclear as it is in *S. cerevisiae*. Neither does the nucleus constrict and separate into daughter nuclei in the neck region between mother cell and bud.

In *L. scottii* the chromatin-containing portion of the nucleus moves into the bud before division. There is partial breakdown of the nuclear envelope and the chromatin divides along a spindle apparatus formed inside the bud within remnants of nuclear envelope. A portion of the nucleus containing the nucleolus is left behind in the mother cell and disintegrates there when the envelope breaks down. After division, the nuclear envelope reforms around daughter nuclei and one daughter moves back to the mother cell. These events have been established on the basis of 3 types of observation: on living cells with phase-contrast microscopy; on cells stained for chromatin and studied with light microscopy; and on glutaraldehyde-fixed cells studied with electron microscopy.

As in *L. scottii*, breakdown of nuclear envelope during division has been reported in several mycelial basidiomycetes. The sequence of chromatin movement into the bud of *L. scottii*, division inside the bud, and return of one daughter nucleus to the mother cell, is closely comparable to the movements of chromatin in and out of clamp connexions known to occur in dikaryotic hyphae of *Polystictus versicolor*. These mitotic similarities are in accord with the taxonomic relationship of *L. scottii* to the basidiomycetes.

Observations on a cytoplasmic organelle located outside interphase nuclei of *L. scottii* have convinced us of its considerable importance during mitosis. This structure consists of 2 globular electron-dense ends joined by a bridge-like middle piece. It accompanies the advancing tips of nuclei which enter the bud. After breakdown of the nuclear envelope, the 2 spherical components of this organelle are seen at opposite poles of the mitotic spindle. Therefore we refer to it as the 'microtubule organizing centre' (MTOC). We have also speculated about a possible role for the organelle in the regulation of nuclear envelope growth, breakdown, and reformation.

INTRODUCTION

Mitosis in budding yeasts, long a controversial subject, is not yet fully understood. It has been shown in *Saccharomyces cerevisiae* that mitosis takes place within an intact nuclear envelope and involves an intranuclear spindle (Robinow & Marak, 1966). However, neither the light microscope nor the electron microscope has yielded much information about the activity of chromosomes during the process. No individual units recognizable as chromosomes can be seen during mitosis and dividing nuclei are, in
fact, less chromatinic than interphase nuclei (see fig. 12 in Robinow & Marak, 1966). Individual chromosomes which form ‘non-classical’ configurations at mitosis have been seen with the light microscope in Lipomyces lipofer (Robinow, 1961). Unpublished electron micrographs of L. lipofer taken in this laboratory have shown that, in this yeast too, mitosis takes place within an intact nuclear envelope and involves an intranuclear spindle. Structures tentatively identified as components of chromosomes were also seen within the nucleus. However, in L. lipofer it is difficult to study the course of mitosis in sections of randomly multiplying cells because there is no obvious correlation of the phases of mitosis with the site and size of buds. Therefore we have searched the literature for accounts of other budding yeasts in which chromosomes might be clearly visible at mitosis.

Such a report was published by Eckstein (1958) about Candida scottii, the yeast described in the present study. Our work with this yeast has not fulfilled our hopes of being able to study the behaviour of chromosomes in a Saccharomyces-like intranuclear division process, but it has, nevertheless, yielded results of considerable interest. We have found that mitosis in C. scottii involves breakdown of the nuclear envelope and differs in several additional ways from the mitosis in ascosporogenous yeasts such as S. cerevisiae, Wickerhamia fluorescens and Schizosaccharomyces pombe, which is achieved by elongation, constriction and equipartition of nuclear components between daughter nuclei without breakdown of the nuclear envelope (Robinow & Marak, 1966; Matile, Moor & Robinow, 1969; McCully & Robinow, 1971). By contrast, the behaviour of the dividing nucleus in C. scottii, described here for the first time in a yeast, is similar to mitotic events in mycelial basidiomycetes.

Until recently C. scottii was considered to be asporogenous, but the finding of a perfect stage (Fell, Statzell, Hunter & Phaff, 1969) has resulted in its reclassification as a heterobasidiomycete in the genus Leucosporidium. Our observations are in accord with this new taxonomic assignation. In conformity with accepted usage, we shall henceforth refer to C. scottii by its new name of Leucosporidium scottii.

**MATERIALS AND METHODS**

**Material**

We have studied a smooth strain of C. scottii Diddens et Lodder No. 4025 in the yeast collection of the Centraalbureau voor Schimmelcultures at Delft, Netherlands. This strain is a subculture of the strain which the collection received from B. Eckstein. Cultures were maintained and propagated for all purposes on a single medium consisting of Difco yeast extract 0.5 g, glucose 20 g and agar 1.5 g per 100 ml of water.

**Phase-contrast microscopy of living cells**

We have used spreading-drop slide cultures in 18% gelatin containing 0.5% yeast extract and 2% glucose prepared according to Robinow & Marak (1966). Best results were obtained with cells that were close to the air space surrounding the flattened gelatin droplet.

Phase-contrast microscopy gave clear-cut information about the early and late phases of mitosis. However, in attempting to record the rapid changes of nuclear form which take place during this process we were handicapped by not having at our disposal an electronic flash illuminating device. The relatively long exposure of 5 s which was necessary has led to a blurring of many features which are more distinct in life than our photographs would suggest.
Fixation and staining for light microscopy

To obtain coverslip preparations of well-preserved cells from randomly growing young cultures on agar we have followed the procedures of inoculation and fixation used by Robinow & Marak (1966). The fixative used was Helly's (mercuric chloride 5 g, potassium dichromate 3 g, water 100 ml) to 10 ml of which 0.6 ml of 37% formaldehyde (formalin) was added just before use. Fixed films were rinsed with 70% ethanol and hydrolysed for 8 min with 1 N HCl at 60 °C. They were then rinsed with water, soaked for a few minutes in 60% glacial acetic acid (v/v), and placed for 30 min in 1% synthetic orcein in 60% acetic acid. Stained films were mounted over a drop of the stain, gently flattened through several layers of blotting paper, sealed with wax or nail varnish and photographed 24 h later.

Microscopy and photography

We used the equipment and materials described in an earlier paper (McCully & Robinow, 1971).

Electron microscopy

Monolayers of cells on agar plates were flooded with cold (4 °C) 3% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2. The cells were scraped off the agar, centrifuged into a pellet and resuspended in fresh cold fixative. Fixation was carried out on ice for 3 h. The glutaraldehyde was washed away with 10-15 changes of cacodylate buffer. Postfixation was in 1% osmium tetroxide dissolved in cacodylate buffer. This fixation was done either at 4 °C (Figs. 5, 14) or at room temperature (all the other electron micrographs) for 4-6 h. Then the cells were washed twice in cacodylate buffer and twice in distilled water before being stained in a 0.5% aqueous solution of uranyl acetate for 2-5 h. Details of incorporation into agar blocks, dehydration, embedding and staining of thin sections are the same as described previously (McCully & Robinow, 1971).

RESULTS

Observations on living budding cells with phase-contrast microscopy

The interphase nucleus of L. scottii, which is spherical or ovoid in shape, consists of a phase-dense nucleolus and an optically transparent chromatin-containing region recognizable by comparison with aceto-orcein-stained preparations described below (Figs. 2:0, 3:0).

At mitosis, a finger-like projection of the chromatin-containing region starts to move into the bud (Fig. 2:15). This projection increases in size for approximately 10 min until it extends about half the length of the bud (Fig. 2:16) while a portion of the nucleus containing the nucleolus remains in the mother cell. Then the nucleus begins to fade from view (Figs. 2:24, 3:25, 3:11, 3:12). Within 1 or 2 min it is completely indistinguishable from the cytoplasm. Nothing more of it can be seen for about 10 min, when 2 small rapidly growing nuclei emerge into view – one in the bud, the other in the mother cell (Figs. 2:35, 3:25, 3:45).

Budding cells of L. scottii usually contain a large vacuole which is evenly filled with phase-dark to-black (not shiny) material in contrast to the vacuoles of S. cerevisiae and W. fluorescens (Robinow & Marak, 1966; Matile et al. 1969). These vacuoles will not concern us further in this paper but it might be mentioned here that they are intensely and metachromatically stained by toluidine blue at a low pH. This may indicate the presence of polyphosphate (Keck & Stich, 1957).
Observations with light microscopy on fixed hydrolysed cells stained with aceto-orcein

The geometry of dividing nuclei in stained Helly-fixed cells is in good agreement with their appearance in life. Stained preparations also bridge the gap of invisibility in living cells between the entry of a large portion of the nucleus into the bud and the sudden simultaneous emergence of daughter nuclei.

Important in our interpretation of mitotic events in fixed cells of *L. scottii* is the observation that buds are distinguishable from mother cells not only by their size but also by their slender shape. Buds have more or less parallel sides with a rounded tip while mother cells are ovoid (Figs. 2-4).

Integration of observations on living and fixed material has convinced us that nuclear division in *L. scottii* passes through the stages described below.

I (Fig. 4, a, b). Interphase nuclei are spherical or ovoid and contain an excentrically placed, relatively large nucleolus. This region is distinct in life and readily stainable with basic dyes applied to unhydrolysed cells but it is free from chromatin and therefore appears as a spherical unstained region in hydrolysed, aceto-orcein-stained preparations. The chromatin which occupies the rest of the nucleus is lightly stained and has a finely granular appearance. In cells with buds, the nucleolus is always positioned in the region of the nucleus that is most distant from the site of bud initiation.

II (Fig. 4, c). A finger-like extension of the chromatin-containing region develops in the region of the nucleus that is close to the base of the bud. This new growth gives the nucleus a pear shape. The rounded base of the pear-shaped nucleus seems to be the same size as the ovoid interphase nucleus (compare b and c in Fig. 4). There is no change in the lightly stained granular appearance of the chromatin at this stage.

III (Fig. 4, d, e). The finger-like extension of the nucleus enters into the bud and becomes progressively longer until it reaches about half the length of the bud. The nucleolus and a portion of the rest of the nucleus remain in the mother cell. At this time the chromatin still stains lightly as in interphase.

IV (Fig. 4, f, g). The chromatin is now completely inside the bud, where it forms a compact rectangular-shaped mass. Between stage III and stage IV there is a considerable change in the appearance of the chromatin. It now stains intensely with aceto-orcein. No individual units recognizable as chromosomes can be seen.

V (Fig. 4, h, i). The chromatin is still completely inside the bud. The rectangular chromatin mass seems to have pulled apart into a dumbbell-shaped configuration. It is deeply stained and no individual chromosomes can be seen.

VI (Fig. 4, j). The central portion of the dumbbell-shaped configuration seems to break and two separate, intensely chromatinic daughter nuclei are seen. These are both still inside the bud.

VII (Fig. 4, k). At this stage only one of the deeply staining daughter nuclei is in the bud. The other daughter has returned to the mother cell.

VIII (Fig. 4, l, m). The daughter nuclei return to their spherical interphase condition with lightly stained granular chromatin surrounding an unstained nucleolus.
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Electron microscopy

The cytoplasm contains a high concentration of ribosomes, several mitochondria, 1-3 prominent vacuoles partially filled with electron-dense material, and electron-transparent regions located just underneath the cytoplasmic membrane (Fig. 5). The latter regions seem to be the sites of some storage material since they are much larger in stationary phase cells than they are in budding cells.

As reported elsewhere (McCully & Bracker, 1972), there are prominent regions of vesicles near the tips of buds in L. scottii (Fig. 5). These vesicles serve as useful bud markers since oblique sections of budding cells often do not reveal the distinctive shape of buds which can be seen with light microscopy.

Buds and mother cells are also recognizable by their respective cell wall characteristics. The outer layers of the mother cell wall seem to rupture at the site of bud initiation and the bud wall is a continuation of only the inner layers of mother cell wall (McCully & Bracker, 1972). Consequently, the wall of the bud usually appears to be slightly thinner than the wall of the mother cell. A collar composed of the ruptured mother cell wall layers can often be detected at the base of the bud (Figs. 8, 13).

The nucleus in interphase cells is spherical or ovoid in shape and consists of 2 regions (Fig. 5). One is a region of low electron density which we can identify as the site of chromatin by comparison with our aceto-orcein-stained preparations. The other is a nucleolus composed of ribosome-like particles dispersed among amorphous electron-dense material. The nucleolus is spherical and seems to occupy one quarter to one third of the total nuclear volume. When buds are present, the nucleolus is always located at the side of the nucleus that is most distant from the bud.

Located in a ribosome-free zone near the outside of the nuclear envelope is a structure which, in view of its later activity during mitosis, we call ‘a microtubule organizing centre’ (MTOC) following Pickett-Heaps (1969) (Fig. 5). This organelle, shown at high magnification in Fig. 7, is composed of 2 electron-dense globular ends and a bridge-like middle part which is of medium electron density. Each of the globular ends is surrounded by a halo of medium electron-dense material which is continuous with the bridge-like middle part. The diameter of each globular end is about 80 nm and the total length of the bipolar organelle during interphase is about 260 nm. In budding cells the position of the MTOC is regularly outside that portion of nucleus which is closest to the base of the growing bud. Although single globular MTOC components were often seen in randomly selected sections, serial sectioning has always revealed another globular component at a different level. We have never observed a structure that we can clearly identify as a single globular MTOC.

The first indications of impending nuclear division are changes in the shape and size of interphase nuclei. They become pear-shaped, with the nucleolus occupying part of the rounded basal portion and the MTOC located outside the pointed tip portion, which extends in the direction of the bud (Fig. 6). The size of the nucleus seems to increase in such a way that the volume of its rounded basal portion remains approximately the same as its original total volume.

The pointed tip of the pear-shaped nucleus later becomes greatly elongated, with
the result that a long protrusion of the nucleus approaches and enters into the bud (Fig. 8). It is accompanied by the MTOC. This bipolar organelle is regularly located on one side of the nuclear projection near its tip (Figs. 8, 9). The bridge of amorphous material joining the 2 globular components of the MTOC is longer than it was in interphase and the ribosome-free area around the organelle now contains cytoplasmic microtubules, small vesicles and profiles of the endoplasmic reticulum (Fig. 9). At this stage the chromatin appears to be almost uniformly of low electron density like the chromatin region of spherical interphase nuclei. This is consistent with our light-microscope observations.

Subsequent mitotic events, which seem to correspond to the period when the nucleus becomes invisible with phase-contrast microscopy, can be summarized as follows. The nuclear envelope on one side of the nuclear projection into the bud becomes discontinuous (Figs. 10, 11, 13). This breakdown may well be the cause of the sudden loss of phase contrast between nucleus and cytoplasm in living cells. The globular ends of the MTOC move into the chromatin region and a spindle-shaped bundle of microtubules forms between them. The chromatin condenses into distinct electron-dense aggregations which are scattered along the spindle (Figs. 10–13). The formation of these aggregations corresponds to the stage at which the chromatin becomes intensely stained with aceto-orcein as described above. The spindle consists of both pole-to-pole and chromosomal fibres. No kinetochore regions of the chromosomes were observed. Chromosomal microtubules simply end in fork-like structures within the chromosomes (Fig. 12).

Before breakdown of the nuclear envelope, at the time when there is a long projection of the nucleus into the bud, a considerable portion of the nucleus including the nucleolus is left in the mother cell. Concurrent with the breakdown of nuclear envelope, the nuclear material in the mother cell seems to disintegrate. Remnants of nuclear envelope (Fig. 10) and what may be a disintegrating nucleolus (Fig. 11) can be seen in the mother cell at the time when a spindle is present in the bud.

Separation of daughter sets of chromosomes seems to progress as the spindle elongates. This elongation has the effect of moving one end of the spindle farther into the bud and the other end of the spindle back towards and into the mother cell. At this time the nuclear envelope seems to lie around the daughter ends of the spindle and along one side of it but the other side of the spindle is open to the cytoplasm (Fig. 14).

We are unclear about the details of how daughter nuclei finally become separated and at what stage an intact nuclear envelope is formed around each of them. Our observations suggest that a considerable portion of the original nuclear envelope is utilized in this process.

We do not know the fate of the single globular MTOC's. At the time when daughter nuclei are moving apart to their respective places in the bud and mother cell and are still connected through a narrow spindle corridor, MTOC's at the ends of the spindle seem to lie inside the new daughter nuclei (Fig. 15). These single MTOC elements may become relocated outside the nucleus before final closure of the envelope or they may disintegrate inside the nucleus.

By the time 2 daughter nuclei are clearly recognizable as separate entities, they are
Fig. 1. Diagrammatic interpretation of mitotic events in *L. scottii*. A, interphase. B, C, D, stages in the movement of a large portion of the nucleus into the bud. It is accompanied by a bipolar microtubule organizing centre and cytoplasmic microtubules. E, F, spindle formation and chromosome separation inside the bud. Note that the nuclear envelope is not present along one side of the spindle. A portion of the nucleus containing the nucleolus has remained in the mother cell and is disintegrating at this stage. G, further elongation of the spindle. One end of the spindle (i.e. a daughter nucleus) is now located in the mother cell. H, reconstruction of intact daughter nuclei.
already spherical with well-formed nucleoli. The chromatin in these nuclei is once again in the diffuse, electron-transparent, interphase condition (Fig. 16). We have searched diligently for any structure recognizable as an MTOC outside daughter nuclei but without any success. However, the possibility that the organelle is present at this stage cannot be completely ruled out.

These events are summarized in the diagram shown in Fig. 1.

DISCUSSION

It is clear from our observations that mitosis in *L. scottii* is different in many ways from mitosis in *S. cerevisiae*. As in *L. scottii*, the dividing nucleus of *S. cerevisiae* elongates and advances into the bud. However, division is then accomplished by constriction in the narrow neck between bud and mother cell with half of the nuclear contents reaching the bud and half remaining in the mother cell. The nucleolus does not break down during mitosis; it stretches out within the elongating nucleus and is evenly distributed between the daughter nuclei. Moreover, division takes place within an intact nuclear envelope (Robinow & Marak, 1966).

A previous report of mitosis in *L. scottii* (*C. scottii*) described a similar process of nuclear elongation and constriction in the region between bud and mother cell (Eckstein, 1958). It was not recognized that all the chromatin enters into the bud before division. The diagrams published by Eckstein show that she observed almost all the chromatin configurations that we have described. Owing, probably, to the dehydration to which she submitted her specimens, the chromatin in her diagrams appears more fragmented than it does in our preparations. This has left her with the impression that she was looking at individual chromosomes. Although Eckstein has tried to arrange her illustrations in a uniform manner with the bud uppermost, some, such as her figs. 11, 16, and 17, are inverted (mother cell uppermost) and correspond to our Figs. 4, 8, 4, and 1 respectively. She considered these configurations to be in the mother cell because she did not recognize that buds invariably have more-parallel sides than mother cells and may sometimes even be larger than the mother cell. Therefore she did not observe that many of the important mitotic events in this yeast are taking place in the bud. We are confident that our interpretation is the correct one since it is based on observations of living cells and is borne out by electron microscopy.

Ultrastructural studies of nuclear division in basidiomycetes have been made by Girbardt (1968) in the dikaryotic hyphae of *Polystictus versicolor*, by Motta (1969), in the rhizomorphs of *Armillaria mellea*, by Lu (1967) in the basidia of *Coprinus lagopus* and by Lerbs & Thielke (1969) and Lerbs (1971) in the basidia of *Coprinus radiatus*. A common feature of division in these fungi is the breakdown of the nuclear envelope. Therefore the breakdown of envelope that we have observed in *L. scottii* is not surprising in view of its reclassification as a heterobasidiomycete (Fell *et al*. 1969).

Certain other features of the mitosis in *L. scottii* strikingly resemble what Girbardt has observed in *P. versicolor* using phase-contrast microscopy (Girbardt, 1961) and electron microscopy (Girbardt, 1968). Since we wish to stress that mitosis in this newly classified heterobasidiomycetous yeast is similar to that observed in other
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basidiomycetes, Girbardt’s observations will be described briefly here and compared with our own.

One of the two nuclei in a dikaryotic cell of *P. versicolor* divides in the hypha and Girbardt has not yet described this mitosis in detail. The other nucleus just prior to mitosis begins to move into the clamp connexion preceded by an electron-dense cytoplasmic structure consisting of 2 globular ends joined by a flat middle piece. This structure is morphologically similar to what we refer to in *L. scottii* as the ‘microtubule organizing centre’. After part of the nucleus has entered the clamp connexion, the nuclear envelope breaks down and much of the rear portion of the nucleus including the nucleolus is left behind and becomes dissolved in the cytoplasm of the hypha. Inside the clamp connexion the 2 globular ends of the electron-dense cytoplasmic structure move apart to form the poles of a bundle of microtubules (the Zentralstrang). The chromatin becomes condensed along this microtubule bundle, and as the microtubules increase in length the 2 daughter sets of chromosomes are separated to opposite poles. At the end of mitosis one daughter nucleus remains in the clamp connexion and the other daughter nucleus moves back to the hypha.

One difference between *P. versicolor* and *L. scottii* is that, in *P. versicolor*, the nuclear envelope which breaks down after the chromatin portion has entered the clamp is later repaired to form a closed vesicle (‘Bläschen’) in which mitosis is carried out, whereas, in *L. scottii*, the spindle is formed and mitosis takes place within a partially torn nuclear envelope which regularly has a large gap on one side of the spindle.

The electron-dense cytoplasmic structures with 2 globular ends seem to have similar roles in the 2 organisms: first in accompanying the tips of nuclei which extend into the bud or the clamp connexion, and secondly in organizing the mitotic spindle. However, in 2 respects the MTOC in *L. scottii* differs from the structure that Girbardt (1968) has called a ‘kinetochore equivalent’ (KCE) in *P. versicolor*. Girbardt finds that this organelle is ‘firmly attached to the nuclear envelope’ and ‘coherent with the chromosomes’ through pores in the nuclear envelope. The corresponding structure in *L. scottii*, although morphologically similar to the KCE in *P. versicolor*, is located close to but apparently not attached to the nuclear envelope. There is no evidence that the organelle in *L. scottii* is attached to chromatin inside the nucleus. During spindle formation the chromosomes are clearly not attached directly to the MTOC’s but are connected to them by chromosomal microtubules. Therefore we do not think that the term ‘kinetochore equivalent’ can be properly applied to the spindle-forming structure in *L. scotti*. We have therefore referred to it as the microtubule organizing centre, a term proposed by Pickett-Heaps (1969) for osmophilic structures which are associated with microtubules.

There is evidence that the organelle in question is capable of doing other things besides serving as a ‘nucleating centre’ for the assembly of microtubules (Tilney, 1968). Girbardt (1968) in his account of mitosis in *P. versicolor* calls it the ‘activity centre’ of the nucleus. Observations on living and stained nuclei of *Ceratocystis fagacearum* and *Fomes annosus* have conveyed the distinct impression that ‘the centriole (MTOC in our terminology) pulls the nucleus through the cytoplasm with the nucleolus lagging behind’ (Wilson & Aist, 1967). At first sight such behaviour seems to be reflected in
the geometry of the nuclei in our micrographs. However, closer examination of our evidence has led us to the conclusion that the interphase nucleus is first carried a short distance from the centre of the mother cell to the neck region, probably by the current of materials that is flowing towards the expanding bud. Starting from this position, it appears to us that the nucleus does not then migrate into the bud; but grows into it. This is immediately suggested by the unchanging shape and dimensions of the lower half of ‘migrating nuclei’. Awareness of this is enhanced by looking at the nuclei of Figs. 2:15, 4, c, d, 6 and 8 with their upper portions covered. It is anyhow not easily seen how the MTOC of *L. scottii* can be pulling a nucleus which it accompanies but to which it is not attached.

It has previously been suggested (Motta, 1969) that the spindle-forming organelle in *Armillaria mellea* might function as a nuclear-membrane organizing body and we have advanced the idea of differential membrane growth between separating MTOC’s as the initial event in elongation of the intact mitotic nucleus of *S. pombe* (McCully & Robinow, 1971). Our observations on *L. scottii* lend support to the view of a possible role for the MTOC in the regulation of localized membrane growth.

We propose that, prior to mitosis, the MTOC could direct the formation of nuclear envelope in that region of the ovoid interphase nucleus that is close to where the MTOC is located (i.e. the region of the ovoid nucleus that is close to the base of the bud). This would result in the formation first of a pear-shaped nucleus and then of a long, sac-shaped one with a rounded base and a neck which extends into the bud. We further propose that the 2 globular MTOC elements of nuclei which have reached the interior of the bud and which are consistently placed on one side of the nuclear extension could cause the nuclear envelope to break down on that side perhaps by temporarily ceasing to perform a putative membrane-maintaining function. After spindle formation the daughter sets of chromosomes move to opposite poles where one of the two original MTOC globular components is present. We think that this organelle may again direct the synthesis (and maintenance?) of new nuclear membrane so that each daughter set of chromosomes becomes enclosed by an intact envelope. Until we know more about the chemical composition of spindle-forming organelles and how membranes are formed, a membrane-regulating role for the spindle-forming organelle in *L. scottii* must remain a hypothesis that cannot be proven.

Our observations on *L. scottii* have led us to conjectures regarding the course of mitosis in certain other yeast genera which we have been able to confirm. The form of mitosis which we have observed in one yeast that is the sporidal phase of a heterobasidiomycete resembles mitosis in hyphal nuclei of basidiomycetes. It follows that the nuclei of other yeasts which have likewise been recognized as phases in the life cycle of heterobasidiomycetes should divide in a way that resembles mitosis in *L. scottii*. This has been found to be the case in *Rhodotorula glutinis* and *Sporobolomyces salmonicolor*. These findings are presented in the second paper of this series (McCully & Robinow, 1972).

In *L. scottii* our observations suggest that after chromosome separation the partially formed daughter nuclei may be pushed apart to their respective places in the bud and mother cell by the elongation of spindle microtubules. Additional evidence for the
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importance of microtubule growth in the separation of daughter nuclei is provided by our observations on Rhodotorula glutinis described in the following paper (McCully & Robinow, 1972).

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REFERENCES


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Figs. 2, 3. Two sets of time-lapse phase-contrast photomicrographs of budding *L. scottii* cells. In each, the numbers at the top state min elapsed since the first picture was taken. \( \times 4500 \)

Fig. 2. At zero minutes the nucleus, containing a phase-dense lens-shaped nucleolus \((n)\) and an optically transparent chromatin region \((chr)\), is seen between a dark vacuole \((v)\) and the bud; 15–16 min later a slender outgrowth from the chromatin-containing portion of the nucleus enters the bud. At minute 24 and for some time after, the nucleus is more or less indistinguishable from the cytoplasm. Daughter nuclei first become visible around minute 35. Only the lower daughter nucleus is shown. The one in the bud is out of focus.

Fig. 3. In this sequence our records note the growth of a bright finger-like extension of the nucleus into the bud. However, at minute 10 the nucleus started to fade from view before it could be photographed and at minute 11 the phase contrast between nucleus and cytoplasm was as shown here. At minute 12 the nuclear material had reached a stage of diffuseness comparable to that seen at minute 24 of Fig. 2. The last two photographs show the newly formed daughter nuclei which were small when they first appeared around minute 25 and had increased in size by minute 45.
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Fig. 4. Nuclear division as seen in fixed hydrolysed cells stained with aceto-orcein. This technique stains the chromatin (chr) and leaves the nucleolus (n) unstained and visible as a white patch within the nucleus. Buds are recognizable because they are usually smaller than mother cells and they have a distinctively more narrow shape. Budding cells of interest in this group have the label placed in the mother cell. Illustrated are: interphase nuclei (a, b); nuclei in the process of entering a bud (c, d, e); condensation of chromatin inside the bud (f, g) (note that the chromatin now becomes more intensely stained); separation of daughter chromatin masses (h, i); movement of one chromatin mass towards (j) and into the mother cell (k); and resumption of interphase in the 2 daughter nuclei (i.e. lightly staining chromatin around a spherical nucleolus (l, m)). $\times 3600$. 

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Fig. 5. Low-power electron micrograph of a mother cell and small bud. The nucleus is located in the middle region of the mother cell (compare with Figs. 2;0 and 3;0). It is spherical and has 2 regions; a chromatin-containing region (chr) of low electron density and an electron-dense nucleolus (n). In the cytoplasm are mitochondria (m), vacuoles (v), electron-transparent regions under the plasma membrane which seem to be the sites of some storage material (sm), and a region of vesicles (ve), near the tip of the bud. Outside the nuclear envelope is a bipolar microtubule organizing centre (mtoc). x 23,000.

Fig. 6. First indications of impending nuclear division (compare with Fig. 4, c). The nucleus is pear-shaped with its pointed tip portion extending towards the bud. Like the spherical interphase nucleus, this nucleus contains an electron-transparent chromatin region (chr) and a nucleolus (n) in which both amorphous electron-dense material and ribosome-like particles can be seen. The microtubule organizing centre (mtoc) is located outside the tip portion of the nucleus. At this magnification the electron-transparent storage material (sm) under the plasma membrane is more clearly visible. x 39,000.

Fig. 7. An MTOC shown at high magnification to reveal its 2 globular electron-dense ends and bridge-like middle part. The nucleus is partially visible at the bottom of the micrograph. Notice that the organelle is located close to but apparently not attached to the nuclear envelope (ne). x 94,000.
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Fig. 8. Further change in the shape of the nucleus prior to nuclear division (compare with Figs. 2:15 and 4, d). Part of the chromatin region (chr) protrudes into the bud while the nucleolus (n) is located in the rounded base of the nucleus which remains in the mother cell. One of the two electron-dense spherical components of the MTOC is visible near the tip of the nucleus. Notice how the cell wall (w) of the bud is thinner than the cell wall of the mother cell. Large arrows at the base of the bud point to the outer layers of mother cell wall which have broken open during bud formation. × 33,000.

Fig. 9. Portion of a protruding nucleus in the bud. A bipolar MTOC is located on one side of the tip. The bridge-like middle part of this organelle is now longer than it was in interphase cells. Vesicles (ve) and endoplasmic reticulum (er) are visible in the ribosome-free area around the MTOC. Cytoplasmic microtubules (mt) extend from the region of the MTOC along one side of the nucleus. Notice the discontinuities in the nuclear envelope near the MTOC (large arrows). These may be initial stages of envelope breakdown prior to spindle formation. × 68,000.
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Figs. 10, 11. Two mitotic spindles (sp) inside buds. In both cells the nuclear envelope is present along the left side of the spindle but the right side of the spindle is open to the cytoplasm. Fig. 10 shows remnants of the nuclear envelope in the mother cell (large arrows). Fig. 11 shows a region of amorphous electron-dense material located in the mother cell (small arrows) which we interpret as a disintegrating nucleolus (n). A single MTOC component is visible at one end of the spindle in Fig. 10; this region (outlined by a rectangle) is shown at higher magnification in Fig. 12. Fig. 10, × 35 000; Fig. 11, × 24 000.

Fig. 12. Spindle microtubules in close association with a single MTOC component. A microtubule running from the region of the MTOC ends in a fork-like structure (large arrow) within a mass of amorphous electron-dense material which we interpret as a chromosome (chr). × 79 500.
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Fig. 13. A mitotic spindle (sp) which has both MTOC components visible at opposite ends. The nuclear envelope is present along the right side of the spindle but the left side is open to the cytoplasm. Masses of amorphous electron-dense material which we interpret as chromosomes (chr) are scattered along the spindle. Large arrows point to regions where the outer wall layers of the mother cell have broken open during bud formation. This feature establishes that the spindle is located inside the bud. $\times 67000$.

Fig. 14. A later stage in spindle elongation. The nuclear envelope is present along the right side of the spindle (sp) but not along the left side. The new daughter nuclei appear as rounded areas at the ends of the spindle. These are partially surrounded by nuclear envelope. A comparison of this micrograph with Fig. 13 seems to indicate that as the spindle tubules elongate, one daughter end is pushed back into the mother cell and the other is pushed farther into the bud. $\times 41000$. 
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Fig. 15. A slightly more advanced stage of daughter-nuclei separation than in Fig. 14. One daughter nucleus is visible in the bud at the top of the micrograph and a glancing section of the other is seen in the mother cell near the bottom of the micrograph. Although it is not apparent in this section, an adjacent section showed that the 2 daughter nuclei are still connected by spindle microtubules and a partially intact channel of nuclear envelope. A small portion of this channel (between arrows) is visible near the bottom of the micrograph. A single spherical MTOC can be seen in association with spindle (sp) microtubules in the daughter nucleus which is located in the bud. Notice how this MTOC seems to be inside the nuclear envelope. \( \times 39000 \).

Fig. 16. At the end of nuclear division a spherical daughter nucleus with a well-defined chromatin region (chr) and nucleolus (n) is located in both the mother cell and the bud. Although several sections of this mother cell and bud were examined, no MTOC was observed in association with either daughter nucleus. \( \times 23000 \).
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