ELECTRON MICROSCOPY OF ULTRATHIN SECTIONS OF *SCHIZOSACCHAROMYCES OCTOSPORUS*

I. Cell Division

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Detailed studies of the process of cell division in yeast, particularly of the genus *Schizosaccharomyces*, have been few (Guilliermond, 1920; Knaysi, 1941), and little is known of the details of this process. Duraiswami (1953) points out that "It would be interesting to study the relationship between karyokinesis and cytokinesis in those yeasts where cell division is effected not by budding but by the formation of a septum in the middle—as for instance in the Schizosaccharomycetes. It is very likely that a close correlation between these two processes exists in these yeasts."

Since the Schizosaccharomycetes appear to divide in a manner which is analogous to that described for bacterial cells, a study by electron microscopy of cytological changes occurring in *Schizosaccharomyces octosporus* during cytokinesis and karyokinesis was carried out not only to elucidate details of the process of cell division, but also to compare and relate these to the process of cell division described for bacterial cells.

MATERIALS AND METHODS

The culture used in these studies was *S. octosporus* strain NRRL Y-854, obtained from Dr. L. J. Wickerham. Cells were routinely grown in a glucose-yeast extract medium (glucose, 1.0 per cent; yeast extract, 2.0 per cent; peptone, 0.5 per cent; KH_2PO_4, 0.1 per cent; and $MgSO_4$, 0.05 per cent). The pH of the medium was adjusted to 7.0 prior to sterilization. When a solid medium was required, 1.5 per cent agar was included. Cells were prepared for study by streaking on agar plates and incubating at 30 C for 24 hr. The culture was then transferred at 12-hr intervals for 2 days. Cells were then washed from the surface of the agar and suspended in 40 ml of liquid medium in 250-ml flasks. The flasks were incubated with vigorous aeration at 30 C and cells were collected at appropriate intervals for examination by light and electron microscopy.

Specimen preparation. The technique used for the preparation of ultrathin sections was a modification of the method described by Hashimoto *et al.* (1958*b*). Cells were fixed in a 1.5 per cent aqueous solution of $KMnO_4$ for 40 min at 4 C. After prolonged treatment with partially polymerized *n*-butyl methacrylate, cells were placed in gelatin capsules and subjected to centrifugation at 2000 × G for 2 min or by allowing them to settle for 4 hr at room temperature. Polymerization was accomplished by placing the capsules in an oven at 60 C for 8 to 12 hr. Specimens were left at room temperature for at least 3 days before sectioning by means of a Porter-Blum microtome equipped with a glass knife. The technique of Satir and Peachey (1958) was employed to decrease compression artifacts. Sections of less than 0.1 μ were picked up on 200-mesh copper grids on which a thin collodion membrane had been mounted and dried. All specimens were examined in an RCA-EMU 2B electron microscope equipped with a 50 or 100 μ objective aperture.

Cytochemical techniques and light microscopy. The nuclear stain of Ganesan and Swaminathan (1958) and Lindegren's modification of the Carnoy-perchloric acid Giemsa staining technique (Lindegren *et al.*, 1956) were employed for the preliminary investigation of nuclear behavior during vegetative cell division. Cell division was also followed by means of phase microscopy.

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Dilute Lugol's solution and Sudan black B were used, respectively, for the demonstration of glycogen and lipoidal inclusions.

RESULTS

Considerable difficulty was encountered in the early attempts to obtain adequately fixed cells which were resistant to explosion during polymerization. These difficulties were overcome by strict adherence to the procedure outlined above. The concentration of $KMnO_4$ and temperature control during fixation and subsequent steps were critical factors. Cells could be fixed for 20, 30, or 40 min, the latter time yielding the most consistent and satisfactory results. Light microscopic observation and measurements revealed that there was less than 10 per cent shrinkage of the cells as a result of the various treatments. It was found that the yeast cell wall was of such low electron density that its detection was difficult in electron micrographs. This difficulty could be partially overcome by treating the sections with lanthanum nitrate (Hashimoto *et al.*, 1958*b*), or by not washing the cells completely free from adhering medium prior to fixation, thereby delineating the outer border of the cell wall. Since many of the cell walls appeared to be refractory to the electron stain, the latter procedure was regularly employed.

Staining of the cells with Giemsa according to the procedure of Ganesan and Swaminathan (1958) revealed the presence and general behavior of the nucleus. Application of Lindegren's modification of the Carnoy-perchloric acid Giemsa staining technique (Lindegren *et al.*, 1956) yielded similar results. The nucleus in most of the cells appeared to be uniformly stained by either technique. However, occasional cells contained deeply stained areas within the nucleus which have been interpreted as being chromosomes by Ganesan and Swaminathan (1958) and Lindegren *et al.* (1956). Alternative interpretations of the significance and nature of these deeply staining areas are discussed by Mundkur (1954), and Hashimoto *et al.* (1959). Although the staining procedures made it possible to follow the general behavior of the nucleus during cell division, detailed observations of structural changes were not possible. Observation of stained cells and phase microscope studies of living cells, indicated that during vegetative division the cells elongated and formed a transverse partition, usually in the medial portion of the cell. The transverse partition appeared to be continuous with the cell wall. Cells then either separated or remained attached, but cell partitioning could proceed without cell separation. Individual cells were generally found to contain a single nucleus in

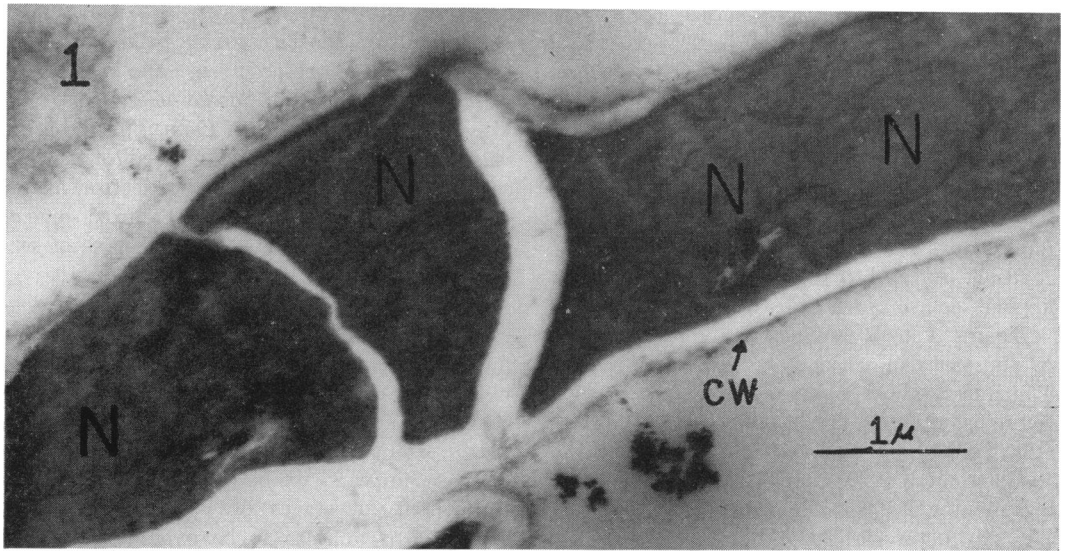


Figure 1. Actively dividing cells. Note the partitioned appearance of the cells, and the nucleus which has elongated and is in the process of constriction. Initiation of cross wall formation is not evident.

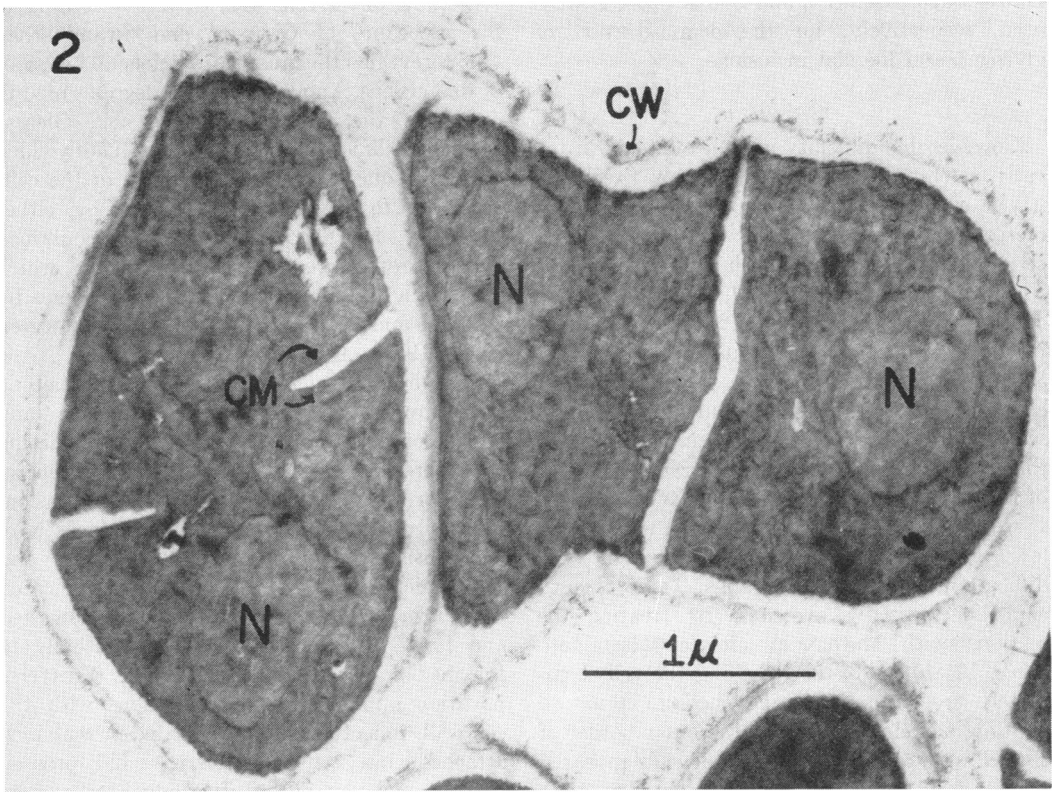


Figure 2. Actively dividing cells illustrating that the plane of division is not always perpendicular to the long axis of the cell. Note the low electron density of the cell wall and the conspicuous nuclei.

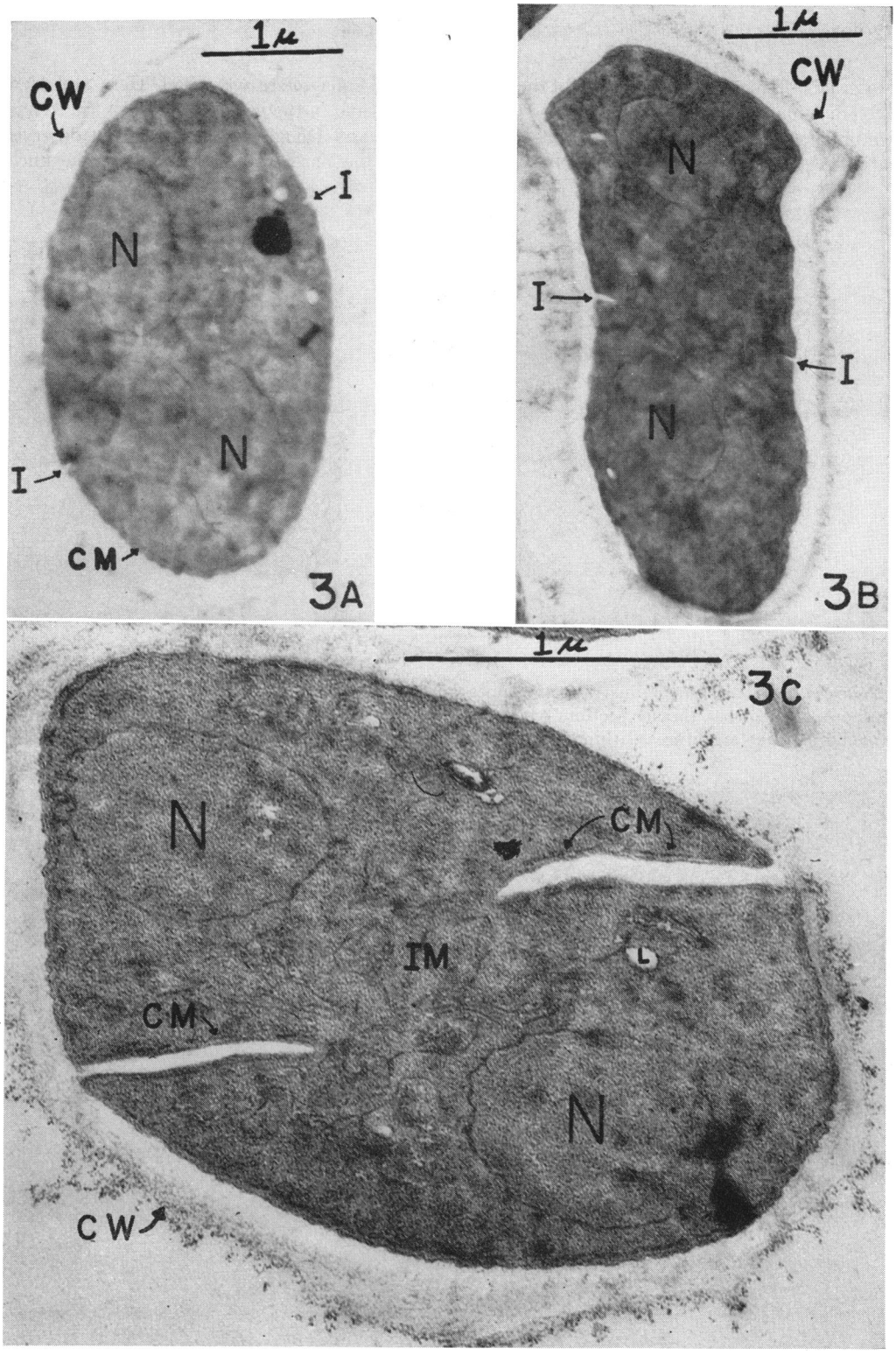
stained preparations. These observations are in agreement with the reports of other investigators (Guilliermond, 1920; Knaysi, 1941; Ganesan and Swaminathan, 1958; Yoneyama, 1958). More detailed observation was beyond the resolution limit of the light microscope. Vacuoles were not seen in young cells observed under phase microscopy, or when stained with dilute Lugol's solution. Sudan black B staining revealed that lipoidal inclusions were absent or few in number.

Figures 1 to 7 are electron micrographs of *S. octosporus* in various stages of cell division. The micrographs illustrate successive stages of cell division based on the preliminary studies by light microscopy and reports in the literature (Guilliermond, 1920; Knaysi, 1941; Ganesan and Swaminathan, 1958; Yoneyama, 1958).

The structure labeled N[3] is considered to be the nucleus on the basis of its behavior during cell division, and the occurrence and behavior of a similar structure in *Saccharomyces cerevisiae* (Hashimoto *et al.*, 1958*a*, *b*; 1959). This structure was found to be present in almost all of the cells sectioned and observed with the electron microscope. The absence of this structure from some of these cells can be ascribed to the plane of sectioning.

Nuclear structure and behavior during cell division. Figures 1, 2, and 3*C* illustrate that the nucleus appears to be internally undifferentiated in electron micrographs, although details of its structure, not observable by light microscopy, are apparent. The nuclear membrane is quite distinct, appears to be double, and persists throughout all stages of vegetative division. The nucleus appears to be ovoid to spherical in shape, in contrast to its lobulate shape in actively dividing cells of *S. cerevisiae* (Hashimoto *et al.*, 1959). The nucleoplasm appears granular and homogenous in texture, with few indications

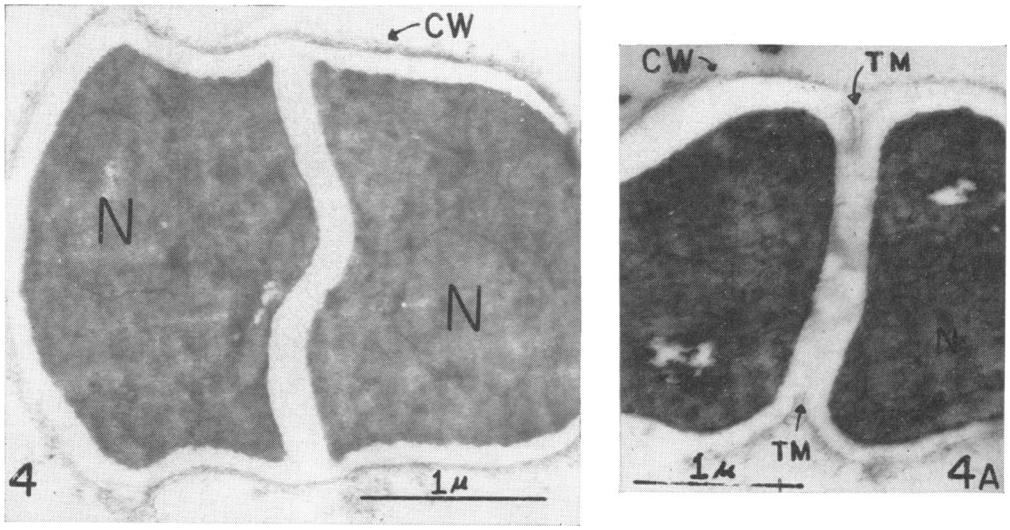
[3] CM = cytoplasmic membrane; CW = cell wall; I = invagination of the cell wall; L = lipoidal inclusion; N = nucleus; TM = transverse cell wall membrane.



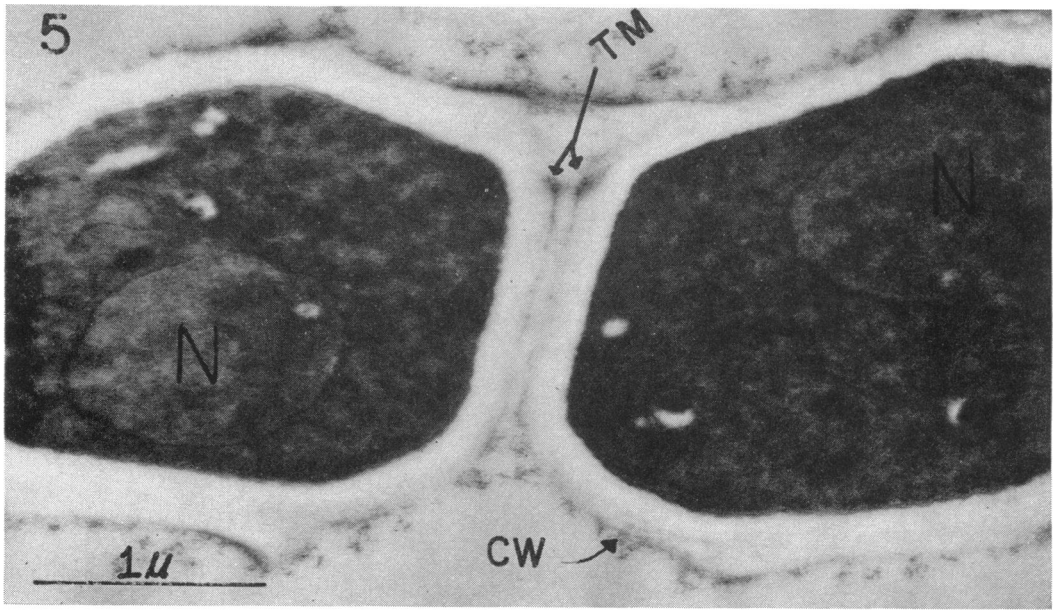
Figures 3A, B, and C. Early and intermediate stages of transverse wall formation. Note in figure 3*A* that the nuclei are separated and cross wall formation is being initiated. Figures 3*B* and 3*C* further illustrate that cross wall formation occurs by the annular centripetal growth of an inner portion of the lateral cell wall. The cytoplasmic membrane is observed to be closely associated with the advancing cross wall in figure 3*C*. Internal membranes can be observed in the area of the yeast cell labeled *IM*.

of internal differentiation during any of the stages of cell division. The pores observed in the nuclear membrane of a variety of animal cells (Kautz and DeMarsh, 1955; Rhodin, 1954; Watson, 1955), *Coccidioides immitis* (O'Hern and Henry, 1956), and in the vegetative cell of *S. cerevisiae* (Agar and Douglas, 1957), were not observed.

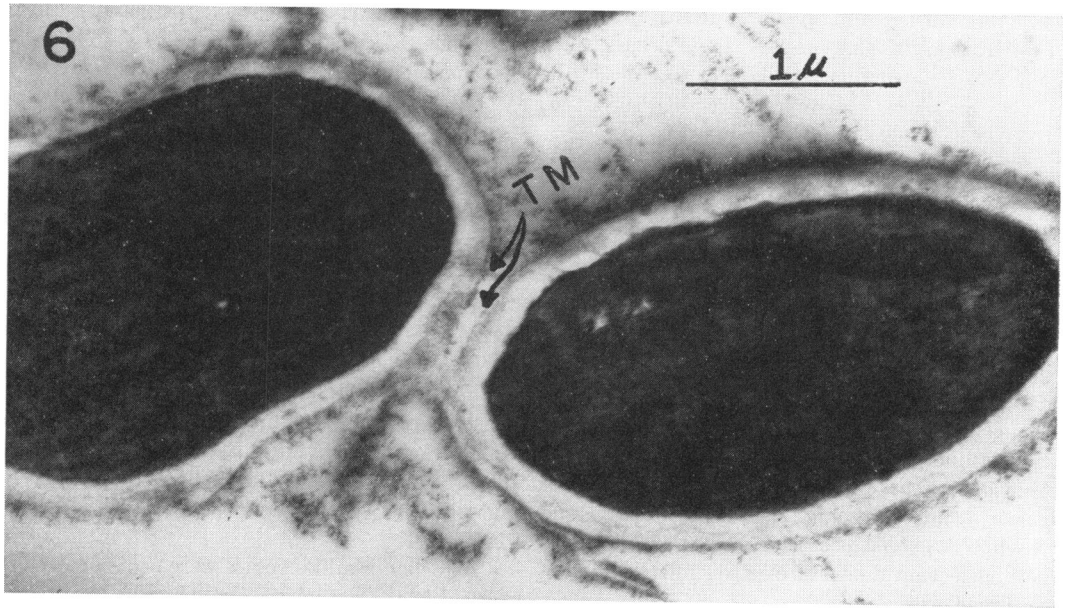
At the onset of nuclear division the nucleus

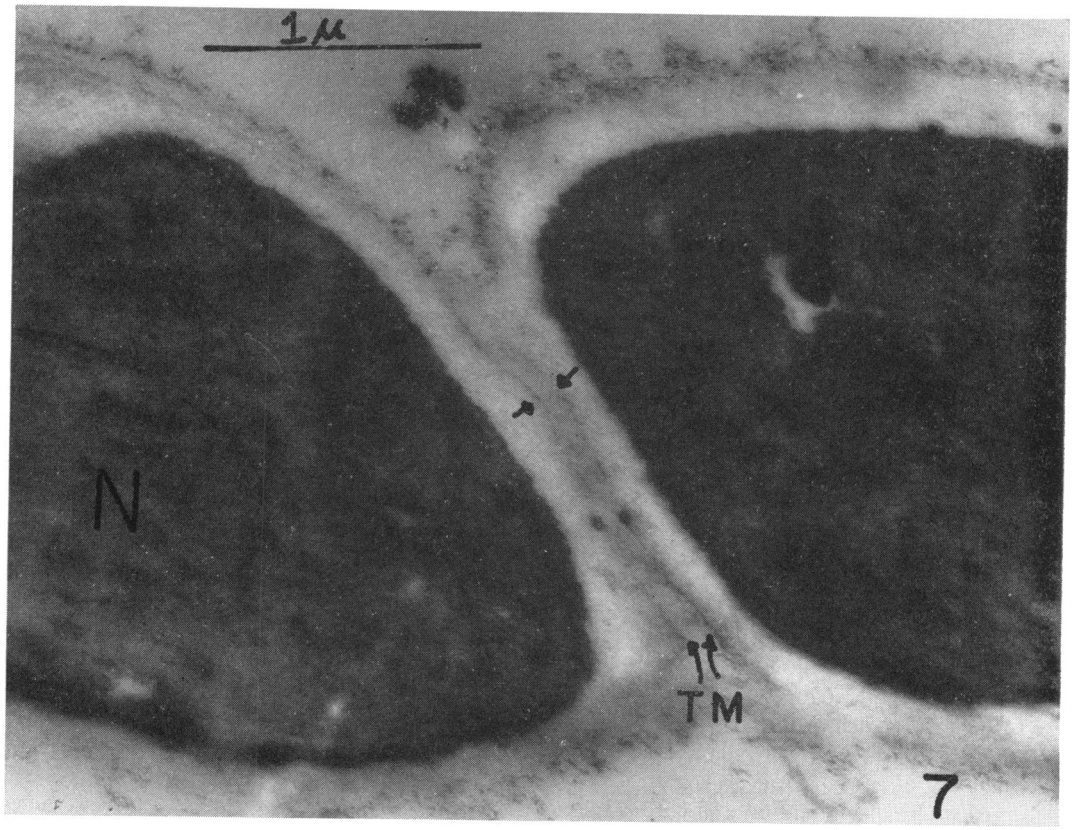


Figures 4 and 4A. Figure 4 illustrates the appearance of the newly formed transverse cross wall. Figure 4A shows a somewhat thicker section, which has been over-exposed in printing. Note the appearance of membrane-like structures within the cross wall.



Figures 5, 6, and 7. These electron micrographs illustrate the development of the membrane-like structures within the connecting cross wall with eventual separation of the daughter cells. Note that these membrane-like structures appear to serve initially as the outer edge of the newly formed portion of the cell wall.





FIGS. 6–7

appears to increase in size and to elongate, taking on a more or less dumbbell appearance (figure 1). Constriction of the nucleus then occurs, presumably in a manner similar to that described in *S. cerevisiae* (Hashimoto *et al.*, 1958*a*, *b*; 1959).

Transverse cell wall formation. During the later stages of nuclear division or after the nuclei are completely separated, transverse cell wall formation occurs by means of the annular centripetal growth of a portion of the inner cell wall into the cytoplasm (figures 2, 3*A*, *B*, *C*). The newly formed cross wall then appears to increase in thickness prior to separation of the daughter cells. The transverse wall, as well as the cell wall, is of low electron density. The cell wall of *S. cerevisiae* (Hashimoto *et al.*, 1959), *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe*, and *Nadsonia fulvescens* (Conti and Naylor, *unpublished observations*) also appear to be nonelectron dense in electron micrographs of sectioned cells.

There are no indications of the prior formation of a cell plate as described in bacteria (Knaysi, 1941, 1949; Chance, 1953; Webb and Clark, 1954; Bisset, 1955; Clark *et al.*, 1957). The position of various cytoplasmic inclusions within the region of cross wall formation, and absence of an observable cross plate supports this contention. The absence of an observable cell plate in dividing cells of *S. octosporus* is in agreement with observations (Knaysi, 1941) on cell division in *S. pombe* by light- and dark-field microscopy. Inward growth of the transverse cell wall is usually not perpendicular to the long axis of the cell, as shown in figures 1 and 2. This, combined with the lag of cell separation after cell division, gives rise to the partitioned appearance of the cells described by various investigators (Guilliermond, 1920; Lodder and Kreger-Van Rij, 1952).

After the cross wall has thickened (figure 4), but prior to cell separation, electron dense, membrane-like areas can be observed within the transverse cell wall (figure 5). The membranes appear to originate in the distal portions of the transverse cell wall. These structures then elongate and thicken, eventually partitioning the connecting cell wall (figures 6 and 7). The inner partition finally disintegrates resulting in complete separation of the two daughter cells in the majority of cases. However, some cells were observed where disintegration was not complete, such cells being attached only by a portion of the cell wall. Attachment in this manner causes the cells to appear in the shape of a "V" which is characteristic of the Schizosaccharomycetes.

Figure 2 reveals that the internal membrane system described in *S. cerevisiae* (Agar and Douglas, 1957) is also present in *S. octosporus*. This membrane system appears to be a normal component of yeast cells, and may be analogous to the endoplasmic reticulum of animal cells (Palade and Porter, 1954; Palade, 1955).

A central vacuole and lipoidal inclusions were regularly observed in sections of vegetative cells of *S. cerevisiae* (Agar and Douglas, 1957; Hashimoto *et al.*, 1959). However, vacuoles appear to be absent and lipoidal inclusions few in number in the actively dividing *S. octosporus*. Vacuoles are regularly observed in cells of *S. octosporus* undergoing sporogenesis (Conti and Naylor, 1959).

Mitochondria-like bodies were occasionally observed in the cytoplasm. In general, these structures appeared to be randomly distributed throughout the cells, in contrast to their mainly peripheral location in *S. cerevisiae* (Agar and Douglas, 1957; Hashimoto *et al.*, 1959). It was noted, however, that these bodies appeared to accumulate in the vicinity of the transverse cell wall during its centripetal growth and remained there while complete separation of the daughter cells occurred. The internal membrane (*cristae mitochondriales*) system described by Palade (1952, 1953) and Sjostrand (1953) as being characteristic of mitochondria was infrequently observed. However, the mitochondria-like bodies were found to be enclosed within a double membrane, and possessed circular or elliptical profiles characteristic of yeast mitochondria (Agar and Douglas, 1957; Hashimoto *et al.*, 1958*b*, 1959).

DISCUSSION

Knaysi (1941) observed cell division in *S. pombe* by both dark- and bright-field microscopy, and reported that a cell plate was not formed, which is in agreement with our results. Knaysi stated further that "cell wall material is deposited at both bases of a cylindrical portion, in the middle of the cell, where chromatin material disappeared," and that there is "no indication of the formation of a single wall with subsequent splitting. . . ." However, later studies by Knaysi (*personal communication*) indicated that trans-

verse cell wall formation occurs in *S. pombe* by the centripetal growth of the cell wall. These studies support the data obtained by electron microscopy, i. e., that cross-wall formation in the Schizosaccharomycetes occurs by the annular centripetal growth of an inner lateral portion of the cell wall. Furthermore, the present studies show that the cytoplasmic membrane remains in close contact with the cell wall and the developing transverse cell wall during the various stages of cytokinesis. Unfortunately, satisfactory pictures were not obtained to show the final stages of the closing of the transverse cell wall.

Comparison of our results with studies by Robinow (1945), Chapman and Hillier (1953), and Chapman (1959) indicates that the process of cytokinesis in bacterial cells and the Schizosaccharomycetes is quite similar. Chapman and Hillier (1953) also observed that after the transverse cell wall is formed it appears to thicken and to divide into two layers. Knaysi (1941) reported that in *Bacillus cereus* "... the portion of the mother-cell wall corresponding to the place of division becomes less and less stainable ... until it withers away liberating the two cells." On the basis of these observations it is tempting to propose that *B. cereus*, and perhaps other bacterial cells, form transverse cell wall membranes, the cells then separating in a manner similar to that observed in *S. octosporus*. The transverse membranes appear to be analogous to the splitting plate observed in budding cells of Histoplasma (Edwards *et al.*, 1959).

The identification of the structure labeled N as the nucleus is in agreement with the views of other workers (DeLamater, 1950; Mundkur, 1954; Agar and Douglas, 1957; Hashimoto *et al.*, 1958*b*, 1959). Examination of the electron micrographs of dividing cells of *S. octosporus* reveal that the nucleus, like that of *S. cerevisiae* (Hashimoto *et al.*, 1959), is surrounded by a limiting membrane which remains intact during nuclear division. The nucleus appears to elongate and divide by constriction, further details of the process remaining beyond the scope of the techniques employed. It is particularly significant that a large central vacuole, such as that regularly found in *S. cerevisiae*, is absent from actively growing vegetative cells of *S. octosporus*. A similar observation has also been reported by Ganesan and Swaminathan (1958).

Although chromosomes have not been observed in electron micrographs of yeast nuclei, this does not in any way establish or indicate that the chromosomes, described by other investigators, are absent. The studies of Moses (1956) and Gibbons and Bradfield (1957) illustrate the difficulties involved in observing chromosomes by means of the ultrathin sectioning technique. The absence of observable chromosomes in the yeast nucleus may be due to one or more of the following factors: (a) The components of the nucleus have similar electron scattering properties, thereby leading to low contrast in the final image. (b) Intranuclear structures are not delineated by membranes. (c) The preparative procedures may result in changing the contents, form, or distribution of intranuclear material. (d) Difficulties in interpretation and observation of the dimensions of structures not well delineated due to the thinness of the section. (e) Chromatin may be distributed uniformly throughout the nucleus in structures beyond the resolution limit of the electron microscope. It therefore should be emphasized that the granular and homogeneous appearance of yeast nuclei in electron micrographs can be reconciled with the reports that chromosomes are present in the yeast nucleus. Studies of alternate thick and thin sections, employing the methods described by Moses (1956) have not been rewarding. Further attempts are now being made to determine the structure and nature of chromatin material in the yeast nucleus.

Although it has been postulated (Mundkur, 1954; Hashimoto *et al.*, 1958*b*, 1959) that chromosomes may be dispersed uniformly throughout the nucleus in the form of submicroscopic chromatinic particles, evidence for this is indirect. Many yeast cytologists consider that the nucleus contains chromosomes comparable in structure to those observed in other organisms, but even among these cytologists, disagreement as to number, size, location, and behavior is apparent. The views of Winge (1951) on this situation are particularly illuminating. His statement that "the numerous technical difficulties involved in yeast cytology have resulted in this field of study being in a most uncertain state" unfortunately still holds true. Progress in the field has been made, however, and most yeast cytologists are now in agreement as to which structure in the yeast cell contains the genetic material. It now seems clear that the yeast vacuole is not an integral part of the nucleus

(DeLamater, 1950; Mundkur, 1954; Agar and Douglas, 1957; Hashimoto *et al.*, 1958*a*, *b*; 1959).

Recent studies of cell division in *Blastomyces dermatitidis* (Bakerspigel, 1957), *Mucor hiemalis* and *Mucor fragilis* (Robinow, 1957*a*), and *Phycomyces blakesleeanus* (Robinow, 1957*b*), are of particular interest since the mode of division of the nuclei in the vegetative parts of these fungi appears to be similar to the process observed in *S. cerevisiae* (Hashimoto *et al.*, 1958*a*, 1959) and *S. octosporus*. The nuclei of these fungi appear to be lacking nuclear membranes in the light microscope whereas the presence of a limiting membrane is established in all the yeasts studied to date by means of ultrathin sectioning and electron microscopy. The comment of Bakerspigel (1957) that "the results during this study suggest that *B. dermatitidis* is another instance of a fungus in which vegetative nuclei do not seem to divide in the classical mitotic manner" is particularly intriguing. Robinow (1957*a*) tentatively proposes that the vegetative nuclei of Phycomyces and Mucor divide by a form of endomitosis. Although this proposal is subject to criticism, Robinow clarifies his position by stating that "endomitosis may seem a rather far-fetched device with which to explain the behavior of Mucor nuclei, but it can hardly be said that more conventional concepts have been more successful in this. Whatever the truth about these nuclei may turn out to be, the contemplation of some of the varieties of chromosome behavior known in the protozoa will probably help us to attain it sooner than Procrustes-like loyalty to the canons of classical mitosis." The validity and applicability of these views to the field of yeast cytology are difficult to question.

SUMMARY

Cell division in *Schizosaccharomyces octosporus* was studied by means of ultrathin sectioning and electron microscopy. It was established that (a) karyokinesis occurs well in advance of cytokinesis; (b) transverse cell wall formation occurs by the annular centripetal growth of an inner lateral portion of the cell wall; (c) the cytoplasmic membrane is closely associated with the cell wall throughout all stages of cytokinesis; (d) after initial formation, the transverse cell wall increases in thickness eventually splitting and completely separating the daughter cells; (e) electron dense membranes are formed which partition the transverse cell wall prior to its splitting, these membranes serving initially as the outer portion of the cell wall; (f) the nucleus is surrounded by a limiting membrane which remains intact during all the observed stages of nuclear and cytoplasmic division; (g) nuclear division can only be described as a process of elongation and constriction, further observations of chromatin behavior being beyond the scope of the techniques used; (h) internal membranes and cytoplasmic inclusions similar in structure to those found in other yeast are present.

REFERENCES

AGAR, H. D. AND DOUGLAS, H. C. 1957 Studies on the cytological structure of yeast: Electron microscopy of thin sections. J. Bacteriol., **73**, 365–375.

BAKERSPIGEL, A. 1957 The structure and mode of division of the nuclei in the yeast cells and mycelium of *Blastomyces dermatitidus*. Can. J. Microbiol., **3**, 923–936.

BISSET, K. A. 1955 *The cytology and life history of bacteria*, 2nd ed. The Williams & Wilkins Co., Baltimore.

CHANCE, H. L. 1953 Cytokinesis in *Gaffkya tetragena*. J. Bacteriol., **65**, 593–595.

CHAPMAN, G. B. 1959 Electron microscopy of ultrathin sections of bacteria. III. Cell wall, cytoplasmic membrane, and nuclear material. J. Bacteriol., **78**, 96–104.

CHAPMAN, G. B. AND HILLIER, J. 1953 Electron microscopy of ultrathin sections of bacteria. I. Cellular division in *Bacillus cereus*. J. Bacteriol., **66**, 362–373.

CLARK, J. B., WEBB, R. B., AND CHANCE, H. L. 1957 The cell plate in bacterial cytokinesis. J. Bacteriol., **73**, 72–76.

CONTI, S. F. AND NAYLOR, H. B. 1959 Conjugation, sporulation and ascospore structure of *Schizosaccharomyces octosporus*. Bacteriol. Proc., **1959**, 50–51.

DELAMATER, E. D. 1950 The nuclear cytology of the vegetative diplophase of *Saccharomyces cerevisiae*. J. Bacteriol., **60**, 321–332.

DURAISWAMI, S. 1953 Studies on the cytology of yeast. VIII. Karyokinesis and cytokinesis in a diploid brewery yeast. La Cellule, **55**, 381–395.

EDWARDS, M. R., HAZEN, E. L., AND EDWARDS, G. A. 1959 The fine structure of yeast-like cells of Histoplasma in culture. J. Gen. Microbiol., **20**, 496–503.

GANESAN, A. T. AND SWAMINATHAN, M. S. 1958

Staining the nucleus in yeasts. Stain Technol., **33,** 115–121.

GIBBONS, I. R. AND BRADFIELD, J. R. G. 1957 The fixation of nuclei in locust testis. In *Electron microscopy,* pp 108–110. Proc. Stockholm Conf., 1956.

GUILLIERMOND, A. 1920 *The yeasts.* Translated by F. W. Tanner. John Wiley & Sons, New York.

HASHIMOTO, T., CONTI, S. F., AND NAYLOR, H. B. 1958*a* Nuclear changes occurring during the bud-formation in *Saccharomyces cerevisiae* as revealed by ultra-thin sectioning. Nature, **181,** 454.

HASHIMOTO, T., CONTI, S. F., AND NAYLOR, H. B. 1958*b* Fine structure of microorganisms. III. Electron microscopy of resting and germinating ascospores of *Saccharomyces cerevisiae.* J. Bacteriol., **76,** 406–416.

HASHIMOTO, T., CONTI., S. F., AND NAYLOR, H. B. 1959 Studies of the fine structure of microorganisms. IV. Observations on budding *Saccharomyces cerevisiae* by light and electron microscopy. J. Bacteriol., **77,** 344–354.

KAUTZ, J. AND DEMARSH, Q. B. 1955 Fine structure of the nuclear membrane in cells from the chick embryo. On the nature of the so-called "pores" in the nuclear membrane. Exptl. Cell Research, **8,** 394–396.

KNAYSI, G. 1941 Observations on the cell division of some yeasts and bacteria. J. Bacteriol., **41,** 141–153.

KNAYSI, G. 1949 Cytology of bacteria. II. Botan. Rev., **15,** 106–151.

LINDEGREN, C. C., WILLIAMS, M. A., AND MCCLARY, D. O. 1956 The distribution of chromatin in budding yeast cells. Antonie van Leeuwenhoek. J. Microbiol. Serol., **22,** 1–20.

LODDER, J. AND KREGER-VAN RIJ, N. J. W. 1952 *The yeasts. A taxonomic study.* Interscience Publishers, Inc., New York.

MOSES, M. J. 1956 Studies on nuclei using correlated chemical, light and electron microscope techniques. J. Biophys. Biochem. Cytol. (Suppl.), **2,** 397–407.

MUNDKUR, B. D. 1954 The nucleus of *Saccharomyces*: A cytological study of a frozen-dried polyploid series. J. Bacteriol., **68,** 514–529.

O'HERN, E. M. AND HENRY, B. S. 1956 A cytological study of *Coccidioides immitis* by electron microscopy. J. Bacteriol., **72,** 632–645.

PALADE, G. E. 1952 The fine structure of mitochondria. Anat. Record, **114,** 427–452.

PALADE, G. E. 1953 An electron microscopy study of the mitochondrial structure. J. Histochem. Cytochem., **1,** 188–211.

PALADE, G. E. 1955 Studies on the endoplasmic reticulum. II. Simple dispositions in cells *in situ.* J. Biophys. Biochem. Cytol., **1,** 567–582.

PALADE, G. E. AND PORTER, K. R. 1954 Studies on the endoplasmic reticulum. I. Its identification in cells *in situ.* J. Exptl. Med., **100,** 641–656.

RHODIN, J. 1954 Correlation of ultrastructural organization and function in the normal and experimentally changed proximal convoluted tubule cells of the mouse kidney. Karolinska Institut, Stockholm, Sweden, Aktiebolaget Godvil.

ROBINOW, C. F. 1945 Nuclear apparatus and cell-structure of rod-shaped bacteria, In Dubos, *The bacterial cell,* p. 353–377. Harvard University Press, Cambridge, Mass.

ROBINOW, C. F. 1957*a* The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. I. *Mucor hiemalis* and *Mucor fragilis.* Can. J. Microbiol., **3,** 771–789.

ROBINOW, C. F. 1957*b* The structure and behavior of the nuclei in spores and growing hyphae of Mucorales. II. *Phycomyces blakesleeanus.* Can. J. Microbiol., **3,** 791–798.

SATIR, P. G. AND PEACHEY, L. D. 1958 Thin sections. II. A simple method for reducing compression artifacts. J. Biophys. Biochem. Cytol., **4,** 345–348.

SJOSTRAND, F. S. 1953 Electron microscopy of mitochondria and cytoplasmic double membranes. Ultra structure of rod-shaped mitochondria. Nature, **171,** 30–31.

WATSON, M. 1955 The nuclear envelope. Its structure and relation to cytoplasmic membranes. J. Biophys. Biochem. Cytol., **1,** 301–314.

WEBB, R. B. AND CLARK, J. B. 1954 Cell division in *Micrococcus pyogenes* var. *aureus.* J. Bacteriol., **67,** 94–97.

WINGE, Ö. 1951 The relation between yeast cytology and genetics. A critique. Compt. rend. trav. lab. Carlsberg. Sér. physiol., **25,** 85–99.

YONEYAMA, M. 1958 Some observations of the multiplication and sporulation of *Schizosaccharomyces versatilis.* Seibutsugakkaishi, **8,** 9–13. Biol. Soc. of Hiroshima Univ., Hiroshima, Japan.