MEIOTIC SPINDLE PLAQUES IN *SACCHAROMYCES CEREVISIAE*

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INTRODUCTION

The plaque-like spindle poles in bakers' yeast have been observed by several investigators, but the relationship of these structures to the nuclear envelope has not been clearly shown Several authors have noted an association of the plaque with a nuclear pore. Robinow and Marak (10) reported that mitotic spindle plaques were "set into" nuclear pores, and according to Moor (7) plaques are seen to be situated in nuclear pores also in freeze-etched mitotic cells Rapport (8) stated that mitotic and meiotic plaques "sit" on nuclear pores, even though the nuclear envelope is too poorly defined in her figures to visualize any association with the plaque.

The difficulty in obtaining adequate fixation in yeast is well known and heretofore has precluded a clear determination of the relationship of the various plaque components to the nuclear envelope. We wish to report on an improved fixation method for yeast and the plaque fine structure as it is revealed by this procedure.

MATERIALS AND METHODS

The diploid strain (S41-arg^4) of *Saccharomyces cerevisiae* used in this investigation was kindly provided by Dr. H. O. Halvorson. Stock cultures were maintained at 4°C on yeast extract peptone medium (YEP) consisting of 2% dextrose, 2% Bacto-peptone (Difco Laboratories, Detroit, Mich.), and 1% yeast extract, and solidified with 1.5% agar. Cells were grown according to the method of Esposito et al. (2). Portions (10 ml) were removed from the sporulation medium at 8, 10, 12, and 14 hr after inoculation. Pelleted cells were washed with distilled water and prepared for electron microscopy. Until the cells adhered together sufficiently to form a pellet, gentle centrifugation after each step was required. This was usually unnecessary once the cells had been postfixed with osmium tetroxide.

Before fixation, sporulating cells were pretreated to obtain spheroplasts according to a modification of the method of Duell et al. (1). All steps in the pretreatment and fixation of cells were carried out at room temperature unless specified otherwise. Harvested, washed cells were suspended in 2.5 vol of medium A (0.1 M β-mercaptoethanol, 0.2 M Tris, 0.02 M EDTA-Na at pH 9) for 15 min at 30°C. The cells were then washed with medium B (0.9 M sorbitol, 0.025 M Na-citrate-phosphate buffer at pH 5.9) and incubated with 1.3 vol of medium B and 0.25 vol glusulase (Endo Laboratories, Inc., Garden City, N. Y.) at 30°C for 10–15 min, with frequent agitation. The cells were then washed twice with medium B and fixed for 30–45 min in Karnovsky's formaldehyde-glutaraldehyde fixative (4) (diluted to half-strength with 0.2 M buffer) containing 0.9 M sorbitol. Phosphate buffer at pH 7 was used throughout the fixation procedure. After several washes in 0.1 M buffer, the cells were stored in buffer overnight at 4°C. They were postfixed in 2% osmium tetroxide in 0.1 M buffer for 1 hr, washed several times in distilled water, and resuspended for 2 hr in a solution of 0.5% uranyl acetate in veronal-acetate buffer (11). The cells were then washed in distilled water, dehydrated through an ethanol series and propylene oxide, and infiltrated with an Epon-Araldite mixture (6). The embedded material was then hardened at 37°C for 3 days and 50°C for 2 days, or at 60°C for 1 day.

Sections were cut on an LKB ultramicrotome (LKB Instruments, Inc., Rockville, Md.) with a DuPont diamond knife (du Pont de Nemours and Company, Inc., Wilmington, Del.). They were picked up on Formvar-coated 200-mesh copper grids over which a thin layer of carbon had been evaporated. The sections were stained for 2 hr at 37°C with uranyl magnesium acetate (3) and 10 min at room temperature with lead citrate (9).

A Siemens Elmiskop I was used at 80 kv with double condenser illumination using a 200 μ condenser aperture and a 50 μ objective aperture. Micrographs were taken on Kodak Electron Image Plates and developed in Kodak HRP.

OBSERVATIONS AND DISCUSSION

With our fixation procedure, the components of the spindle plaque, microtubules, and the nuclear envelope are all well defined. In addition, the plasma membrane, cytoplasmic ribosomes, and mitochondria are well preserved.

Figs. 1 *a*, *b* show a Meiosis I spindle plaque in profile. It is embedded within an apparent discontinuity in the nuclear envelope and has the appearance of an enlarged nuclear pore with dense material on both of its surfaces.

Three plaque components can be observed in cross-section, and these are named according to their location with respect to the nuclear envelope. The region resembling a nuclear pore is the central zone. Generally, it is the narrowest and most darkly stained plaque component. Its edge is formed by the junction of the two leaflets of the nuclear envelope (Fig. 1 *c*). This junction is also observable at the edge of a nuclear pore in cross-section (Fig. 1 *d*). An accumulation of dense material on the cytoplasmic side of the central zone is designated the outer zone. Between the outer and central zones, a region of low electron opacity is frequently observed. The plaque component on the inside of the nuclear envelope is called the inner zone. It is from this zone that the spindle microtubules appear to emanate. This zone is also frequently separated from the central zone by a region of low electron opacity.

Bundles of intranuclear spindle microtubules approximately 200 A in diameter are present and terminate at the inner zone of the plaque. Each microtubule in Figs. 1 *a*, *b* appears to end at a narrow dense line at the distal side of the inner zone. So far, we have not seen any convincing evidence for microtubules emanating from the outer zone of the plaque. In addition to the spindle tubules, narrower tubules approximately 120 A in diameter, often arranged in parallel bundles, are frequently observed within the nucleus. It is not

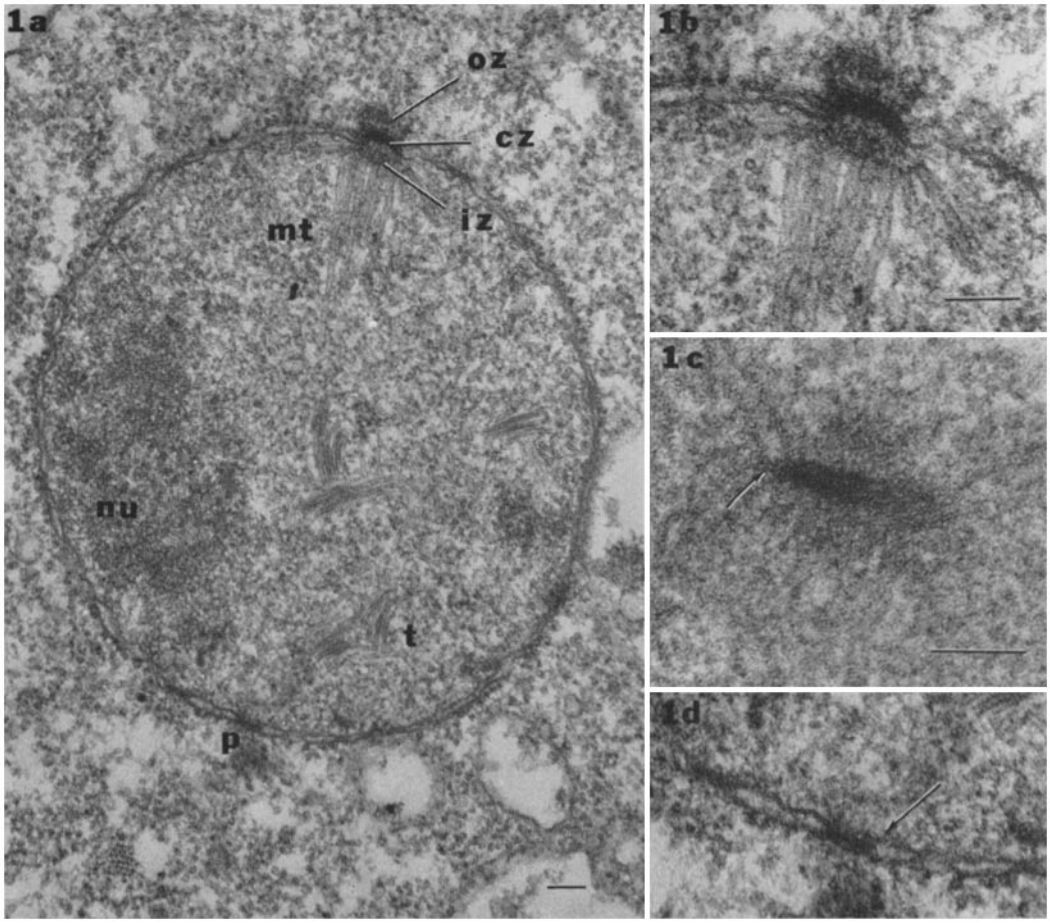

FIGURE 1 (*a*, *b*) Cross-section through a Meiosis I spindle plaque. (*a*) The plaque is composed of an inner zone (*iz*), an outer zone (*oz*), and a more dense central zone (*cz*) which resembles an enlarged nuclear pore. Both the inner and outer zones are separated from the central zone by a region of lower electron opacity. Intranuclear microtubules (*mt*) radiate from the inner zone. Bundles of narrower tubules (*t*) are scattered within the nucleus. Nuclear pores (*p*) and the nucleolus (*nu*) are prominent. × 48,000. (*b*) Enlarged view of the plaque shown in Fig. 1 *a*. × 90,000. (*c*) Another Meiosis I spindle plaque in profile At the edge of the central zone, the continuity of the two leaflets of the nuclear envelope can be seen (arrow). ×124,000. (*d*) Enlarged view of the nuclear pore labeled in Fig. 1 *a*. Its edge is also formed by the junction of the two leaflets of the nuclear envelope (arrow) × 90,000. Figs. 1 *a–d*, calibration lines represent 0.1 μ.

known whether these tubules have any relationship to spindle microtubules.

Our description of meiotic plaque ultrastructure differs somewhat from that of Moens and Rapport (5) mainly because the nuclear envelope is better preserved with our fixation procedures. These authors observed that both meiotic divisions occur within one nucleus. By serial sectioning nuclei, they were able to detect the presence of two spindles (and four plaques) within the nucleus during Meiosis II. Fig. 2 *a* shows a Meiosis II nucleus in profile, and three out of the four plaques are apparent. Plaque 1 in this figure is shown in Figs 2 *b* and *c* as it appeared in the next two sections The fine structure of the plaques illustrated in these figures is more readily observed because the cell membrane is broken and the cytoplasm around the plaque is dispersed We also observed that the dense outer zone is larger and more darkly stained than the inner zone during the second meiotic division. It appears somewhat curved and tends to surround the rest of the plaque. The area

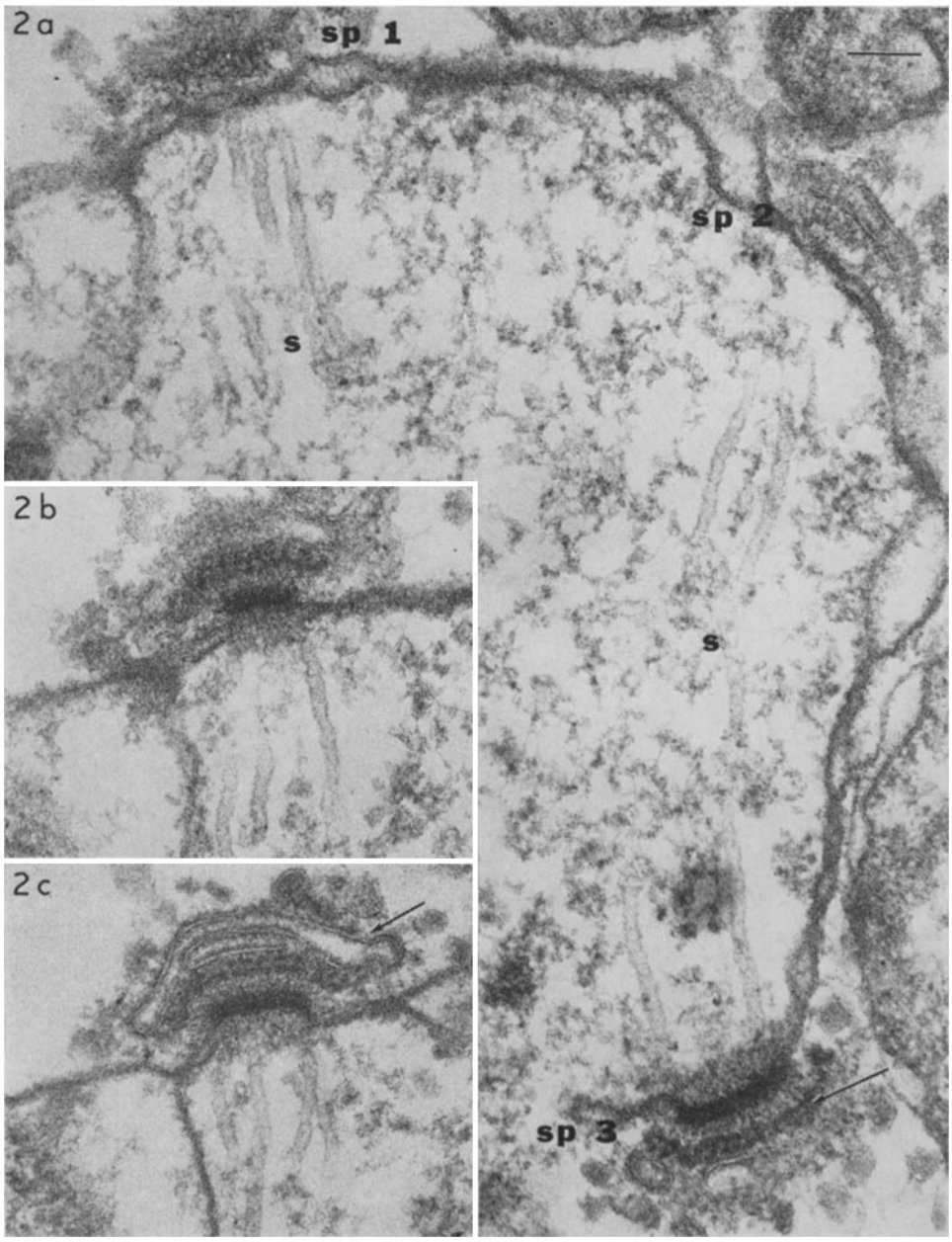

FIGURE 2 (*a–c*) Meiosis II plaques in profile. (*a*) Three of the four plaques present in a Meiosis II nucleus are visible in this section. The components of spindle plaque 3 (*sp 3*) are most clearly seen. The outer zone of the plaque (arrow) is larger than in Meiosis I and it is noticeably denser than the inner zone. The material comprising the region of low electron opacity between the outer and central zones of this plaque, as well as the outer zone itself, appears to be oriented in parallel arrays perpendicular to the surface of the nucleus. Only the edges of spindle plaques 1 (*sp 1*) and 2 (*sp 2*) are viewed in this section. Microtubules comprising both spindles (*s*) are present. × 84,000. (*b*, *c*) Spindle plaque 1 as observed in the next two sections. × 84,000. Flattened, membranous vesicles (arrow) appressed to the outer zone of the plaque can be observed in Fig. 2 *c*. Figs. 2 *a–c*, calibration lines represent 0.1 μ.

of lower electron opacity between the nuclear envelope and the outer zone, as well as the outer zone itself, seems to contain a periodic array of material perpendicular to the surface of the nuclear membrane. Microtubules comprising the two spindles can be seen.

In Figs. 2 *a* and *c*, flattened membrane-enclosed vesicles are visible appressed to the outer zone of the Meiosis II plaques. Membranous vesicles associated with the plaque outer zone at this time have also been seen in intact cells. Similar structures have been previously noted and are believed to be involved in the formation of the prospore wall (5).

Yeast cells have generally proved refractory to ultrastructural investigation. A major difficulty was attributable to poor penetration of fixatives through the cell wall. Enzymatic pretreatment of the cells to form spheroplasts, however, followed by double aldehyde and osmium fixation greatly improves the quality of preservation obtainable. It now seems feasible to undertake an ultrastructural study of mutants defective in nuclear division.

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REFERENCES

1. Duell, E. A., S. Inoue, and M. F. Utter. 1964. Isolation properties of intact mitochondria from spheroplasts of yeast. *J. Bacteriol.* **88**:1762.
2. Esposito, M. S., R. E. Esposito, M. Arnaud, and H. O. Halvorson. 1969. Acetate utilization and macromolecular synthesis during sporulation of yeast. *J. Bacteriol.* **100**:180.
3. Frasca, J. M., and V. R. Parks. 1965. A routine technique for double staining ultrathin sections using uranyl and lead salts. *J. Cell Biol.* **25**:157.
4. Karnovsky, J. J. 1967. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**:137*a* (Abstr.)
5. Moens, P. B., and E. Rapport. 1971. Spindles, spindle plaques, and intranuclear meiosis in the yeast *Saccharomyces cerevisiae* (Hansen). *J. Cell Biol.* **50**:344.
6. Mollenhauer, H. H. 1964. Plastic embedding mixtures for use in electron microscopy. *Stain Technol.* **39**:111.
7. Moor, H. 1966. Ultrastrukturen im Zellkern der Bäckerhefe. *J. Cell Biol.* **29**:153.
8. Rapport, E. 1971. Some fine structure features of meiosis in the yeast *Saccharomyces cerevisiae*. *Can. J. Genet. Cytol.* **13**:55.
9. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.
10. Robinow, C. F., and J. Marak. 1966. A fiber apparatus in the nucleus of the yeast cell. *J. Cell Biol.* **29**:129.
11. Ryter, A. and E. Kellenberger. 1958. Etude au microscope electronique de plasmas contenant de l'acide desoxyribonucleique. I. Les nucleotides des bacteries en croissance active. *Z. Naturforsch.* **13b**:597.