Behavior of Spindles and Spindle Plaques in the Cell Cycle and Conjugation of Saccharomyces cerevisiae

BRECK BYERS* AND LORETTA GOETSC

Department of Genetics, University of Washington, Seattle, Washington 98195

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The interdependence of spindle plaque behavior with other aspects of cell division and conjugation in Saccharomyces cerevisiae has been investigated. Three forms of the spindle plaque appear sequentially before the formation of the complete, intranuclear spindle. The single plaque is present initially in the mitotic cycle; it becomes transformed into a satellite-bearing single plaque during the latter part of G1. Subsequently, plaque duplication yields the double plaque characteristic of the early phase of budding, which coincides with the period of chromosome replication (S). The eventual separation of these plaques to form a complete spindle, with a single plaque at each pole, is nearly coincident with the completion of S. The form of the plaque differs in two independent cases of G1 arrest: the single plaque is found in a cell in stationary arrest of growth, whereas a cell arrested by mating factors in preparation for conjugation contains a satellite-bearing single plaque. The latter form is retained during zygote formation, where it serves as the initial site of fusion of each prezygotic nucleus with the other. This fusion results in the formation of a single zygotic nucleus with a satellite-bearing single plaque, which is subsequently transformed into a double plaque as the zygote buds. The double plaque is situated adjacent to the site of bud emergence in both vegetative cells and zygotes.

The cell division cycle of Saccharomyces cerevisiae involves the progression of events required for nuclear division concomitantly with those involved in bud development. Cytological analysis has revealed that the two spindle plaques, densely staining discoidal structures that are situated in the nuclear envelope and serve as the poles of the intranuclear spindle, arise by duplication of a single spindle plaque by the time of early bud development (8). Chromosomal deoxyribonucleic acid replication occurs at this same phase of the cell cycle (19).

Functional interdependence of these events is suggested by the behavior of temperature-sensitive mutants of the cell division cycle characterized by Hartwell and his colleagues (6). Strains mutant in cdc genes become arrested at particular stages of the cycle upon transfer to the restrictive temperature. Electron microscopy of several strains has revealed that these specific stages of arrest are reflected in similarly specific conformations of the spindle and spindle plaques (1). All budded strains were found to have undergone duplication of the spindle plaques. Among the budded strains, those mutant in cdc 4 were unique in two pertinent features. On one hand, the spindle plaques remained in a side-by-side configuration (or double plaque) rather than separating to form a complete spindle. In addition, these cells retained a capacity for repeated budding in the absence of nuclear division.

These observations suggest that the spindle plaques may play a role in the control of other cellular events, particularly of bud emergence. We therefore have undertaken an electron microscopy examination of normal vegetative growth and conjugation to determine how these events may be interrelated.

MATERIALS AND METHODS

Strains and media. Haploid strains A364 A a and S2072 a of Saccharomyces cerevisiae were obtained from L. H. Hartwell, diploid strains 2681-9A a/a and 1422-4C a/a were provided by D. C. Hawthorne, and diploid strain AP-1 a/a by A. K. Hopper. All strains have several auxotrophic requirements but were grown on complete media for all experiments described here. YEpd medium consists of 1% yeast extract, 2% peptone (Difco), and 2% glucose; 1.5% agar was added for plates. PSP2 medium for growth in acetate contains 0.67% yeast nitrogen base, 0.1% yeast extract, 1% potassium phthalate, 1% potassium acetate, and 0.004% each of adenine and uracil; the medium was adjusted to pH 5.4 with potassium hydroxide.
Assay of budding in logarithmic growth. Because it was necessary to determine the growth rate of buds under conditions identical to those employed for cultures fixed for electron microscopy, samples of the asynchronous cultures grown in PSP2 were fixed in 3% glutaraldehyde in 0.1 M cacodylate, pH 6.8, and were disaggregated by ultrasonication. Portions were then photographed by phase contrast microscopy and the films were projected for measurement in a Nikon Profile Projector. Bud and cell diameters for 200 cells from each sample were determined by the criteria of Williamson (19). The frequency distribution of these values was plotted (Fig. 1), thereby permitting determination of the relative age in the cell cycle of an average cell.

Mating. The logarithmically growing cultures to be mated were mixed on membrane filters (Millipore Corp., 0.45-μm pore diameter) at a density of 10⁷ cells of each mating type per filter of 25-mm diameter (4). These filters were then incubated on the surface of YEPD plates until cells were recovered to be scored and fixed for electron microscopy.

Preconjugatory stages were kindly provided by L. H. Hartwell and Linda Wilkinson. Haploid strain A364A α was blocked at this stage by the addition of α mating factor (4) and S2072 α by a similar factor from a culture of α mating type cells (18).

Electron microscopy. Cells were fixed in 3% glutaraldehyde in a buffer containing 0.1 M cacodylate, pH 6.8, and 5 mM CaCl₂ at 20 C for 30 min and then at 0 C for about 16 h. Fixation of cultures in stationary phase was preceded by treatment for 10 min at 20 C with 0.1 M β-mercaptoethanol in 0.02 M ethylenediaminetetraacetic acid (Sigma) and 0.2 M tris(hydroxymethyl)aminomethane-hydrochloride (Sigma), pH 8.1, to facilitate later removal of walls. After glutaraldehyde fixation, walls were removed by incubation of the washed cells in 1% volume glusulase (Endo Laboratories) in 0.2 M phosphate-citrate buffer, pH 5.8. Cells were subsequently postfixed for 60 min at 0 C in 2% osmium tetroxide in 0.1 M cacodylate, pH 6.8, and 5 mM calcium chloride, and then rinsed with water, treated for 60 min at 20 C with 2% aqueous uranyl acetate, dehydrated, and embedded in Spurr resin (15). Blocks were serially sectioned on a Sorvall MT-2 ultramicrotome; the ribbons were picked up on formvar films on 1 by 2-mm oval single hole grids, stained successively with uranyl acetate and lead citrate, and viewed in a Philips EM 300 electron microscope. Cellular structures were measured in electron micrographs at a total magnification of 10,000 diameters.

RESULTS

Spindles and spindle plaques in the division cycle. Our initial observations on the morphology of logarithmically growing yeast largely confirmed earlier studies (8, 9, 12) and are summarized diagrammatically in Fig. 2a to f. Unbudded yeast cells possess a single, discoidal dense structure, the spindle plaque, embedded in the nuclear envelope; microtubules of the intranuclear spindle end abruptly on its intranuclear surface. Near the time of earliest bud emergence, the plaque has become duplicated such that two similar plaques lie side by side in the nuclear envelope, separated from one another by a specialized connecting structure, the plaque bridge. This configuration of side-by-side plaques (9), which we shall henceforth term a double plaque, persists during the earlier phase of bud enlargement and eventually separates into two distinct plaques which form the poles of a complete intranuclear spindle. The spindle rapidly achieves a stable length slightly less than the average diameter of the nucleus, its poles usually lying within cytoplasmic indentations of the nuclear envelope. This length of spindle persists until the bud reaches a size similar to that of the mother cell. The nucleus then migrates into the neck between the cell and bud. Renewed elongation of the spindle then occurs, apparently forcing the poles of the nucleus into the distal portions of the cell and bud (8). The nucleus subsequently pinches apart within the neck and the cytoplasm is divided by a process involving the fusion of cytoplasmic vesicles. Cytokinesis is completed by the deposition of wall material in this region (8).

From among these events we have concentrated our attention on the temporal relation-

**Fig. 1.** Cumulative distribution of bud size (b/c, bud diameter/cell diameter) in asynchronous populations of haploid (A364A) and diploid (AP-I) cells growing in medium PSP2.
ship between plaque duplication and bud emergence, attempting to determine whether the order of events is consistent with a functional interaction between these processes. Because we are constrained to observe the structure of fixed, rather than living, cells, the temporal sequence must be deduced from morphological clues in cells of an asynchronous population. The extent of progress through the budding cycle by an individual cell is indicated by the ratio of diameters of the bud and cell (19). Figure 1 shows a cumulative frequency distribution of this ratio, determined from measurements of light micrographs. The dotted line represents the increase in relative bud-to-cell diameter by an average cell as it progresses through its cell division cycle. This determination is less precise during the early phase of bud emergence when buds too small to be scored by light microscopy would be resolved by electron microscopy. Therefore, bud emergence actually begins slightly earlier than the smallest nonzero ratios recorded. Thereafter, a nearly linear rise in the ratio of bud to cell diameter provides an index of cell age in the budding cycle.

Samples from the same cultures were embedded and sectioned for electron microscopy. Representative stages were selected from those cells in which the spindles and spindle plaques were adequately oriented for identification of their forms and dimensions. These features were compared with the ratio of bud to cell diameters (averaged between the maximal dimension found in the serial set of images and the dimension perpendicular to it).

We find three classes of plaques (represented by solid horizontal structures in Fig. 3) in these cells: (I) single, (II) satellite-bearing single, and (III) double plaques. The single plaque (Fig. 3-I and 4a to b) consists of a dense disk with an adjacent densely staining inflected membrane of the nuclear envelope, the half-bridge, on one side; microtubules (paired vertical lines in Fig. 3) of the spindle extend into the nucleus (stippled in Fig. 3) from the plaque proper and extranuclear microtubules are occasionally found to extend outward from an outer layer of material similar in appearance to the plaque but of lesser thickness and diameter (the outer plaque). Single plaques are found in many unbudded cells, particularly those which appear to have completed cytokinesis most re-

![Fig. 2. Diagram indicating the behavior of spindle plaques and satellites (both stippled), half-bridges (bold lines), and microtubules (straight lines) during the budding cycle and the conjugation process of Saccharomyces cerevisiae in ideal cross-sectional views (except perspective views of nuclear fusion during conjugation in i-k).](attachment:image)

![Fig. 3. Diagrams of sections through the fundamental forms of the spindle plaque present in successive stages of the cell division cycle: (I) single plaque, (II) satellite-bearing single plaque (which persists during conjugation), (III) double plaque. The complete spindle (CS) bears a typical single plaque at each pole.](attachment:image)
cently as indicated by the irregular contour of the cell surface at the glucosylase-resistant bud scar.

In cells which have lost the irregular contour and are therefore apparently older, a satellite-bearing single plaque (Fig. 3-II and 4c to d) is found. This form has all the features of the simple single plaque but in addition carries at the opposite end of the half-bridge a sphere of dense amorphous material similar in appearance to plaque material. This satellite differs from a true second plaque—that is, from the other half of a double plaque—by three criteria. First, it is situated wholly on the cytoplasmic side of the half-bridge and contiguous nuclear envelope, not embedded in it as is a true plaque. Second, and probably deriving from the first attribute, there are no spindle microtubules attached to its intranuclear side. Third, there is no outer plaque—that is, no outer layer of dense material on the cytoplasmic side of the satellite. In addition, it is usually of smaller dimensions. As indicated in Fig. 3, this form of the plaque is also found in conjugation, the details of which are presented later in this report.

The double plaque (Fig. 3-III and 5b to c) appears to consist of two single plaques which share a common bridge, rather than each bearing a separate half-bridge. Both components usually display an outer plaque and bear spindle microtubules on the intranuclear surface. The two halves of the double plaque differ, however, from typical single plaques with re-

![Fig. 4. Detailed electron micrographs of spindle plaques from the earlier phases of the division cycle in logarithmically growing diploid cells. (a, b) Serial sections of a single plaque. (c, d) Serial sections of a satellite-bearing single plaque. CM, Cytoplasmic microtubule; HBr, half-bridge; OP, outer plaque; S, satellite; SM, spindle microtubule; SP, spindle plaque. Scale lines on this and subsequent plates represent 0.2 μm.](image-url)
gard to their association with extranuclear microtubules. Such microtubules are attached not only to the outer plaque but also to the central region of the external surface of the bridge.

Double plaques were observed exclusively in cells with small buds, never in unbudded cells. This determination depended upon a more stringent definition of early budding than that applied for light microscopy, where the smallest buds are not resolved. In the electron microscope it is possible to recognize the earliest bud as a slight surface evagination containing several vesicles (usually 40 to 60 nm, but sometimes as much as 250 nm, in diameter; 10). By this criterion for budding, observations on a growing diploid culture have revealed the lack of double plaques in 20 unbudded cells and their presence in all of 20 budded cells with ratios of

Fig. 5. Diploid cells in early stages of budding. (a) Observations on adjacent sections demonstrated this spindle plaque (SP) to be a double plaque with cytoplasmic microtubules (CM) directed into the vesicle-laden early bud. M, Mitochondrion. (b, c) Serial sections of another cell show both halves of the double plaque and cytoplasmic microtubules (CM) attached to the bridge.
bud diameter/cell diameter of less than 0.4. Assuming an equal distribution of cases throughout the age distribution of these unbudded and budded phases (each being about 20% of the cell cycle), the elapsed time between plaque duplication and bud emergence would be no more than about 2% of the cycle, regardless of their order of occurrence.

The persistence of the double plaque during bud growth was determined from the ratio of bud diameter/cell diameter in electron micrographs (Fig. 6). The early limit of its appearance, as indicated by the presence of a bud, is obscured by the lack of precise timing of this parameter by light microscopy. The data demonstrate, however, that the double plaque remains until the ratio of bud to parent cell diameter is about 0.35 in the haploid and 0.40 in the diploid, after which a complete spindle forms. By reference to the frequency distribution in Fig. 1, we may then deduce that these cells retain double plaques during at least 30% of the budded phase of the cycle.

As the bud enlarges further, the plaques separate rapidly from one another to form a complete spindle (Fig. 2d), which retains a stable length of 0.95 ± 0.10 μm for about 65% of the budded phase. This length persists until the bud and cell are of equal diameter; by this time the nucleus has moved into the neck connecting them (Fig. 7). Then the spindle elongates rapidly (within about 5% of the cell cycle) to a length of 6 to 8 μm. Nuclear division and cytokinesis then ensue as described by Matile et al. (8). The plaques of all stages with complete spindles retain the morphology of single plaques (Fig. 3-CS), including the attached half-bridge and outer plaque with occasional attached extranuclear microtubules.

Association of the double plaque with the budding site. In making these observations we noticed that the double plaque was spatially as well as temporally associated with the early bud. To quantify the spatial relationship, we made measurements on these electron micrographs of the distance between the center of the double plaque and the center of the neck joining the early bud to the mother cell. For comparison we measured the distance from the center of the neck to the nuclear midpoint (the intersection of the greatest diameter of the

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**Fig. 6.** Correlation between the relative bud size (b/c, bud diameter/cell diameter) and the stage of spindle development. The dashed line demonstrates two persistent phases (the double plaque [DP] and a spindle about 1 μm long) before terminal elongation.

**Fig. 7.** A complete spindle beginning its terminal phase of elongation in a nucleus lying within the neck of a budded diploid cell. CM, Cytoplasmic microtubule; HBr, half bridge; SM, spindle microtubule; SP, spindle plaque.
nucleus with its perpendicular diameter). All cells analyzed (a total of 34) displayed a smaller distance from the neck to the double plaque than to the nuclear midpoint. A set of 13 haploids showed an average neck-to-plaque distance of 1.50 μm versus a neck-to-nuclear midpoint distance of 1.86 μm. A set of 21 diploids yielded values of 1.26 and 1.95 μm, respectively. Therefore, the double plaque consistently resides on the side of the nucleus nearer the neck.

The orientation of the plaque toward the bud was verified by measurements of the angle between two straight lines from the bridge of the double plaque, one directed toward the center of the neck and the other drawn perpendicular to the plane of the bridge (Fig. 8). These data clearly confirm a favored orientation of the double plaque toward the neck of the early bud. We examined the possibility that this association was maintained most strongly during the earlier phase of bud emergence, but we found no systematic variation in the magnitude of this angle with the age (relative diameter) of the buds. It is clear, therefore, that the double plaque is oriented toward the bud throughout this phase of the cycle.

A physical association between the double plaque and the early bud is further indicated by the frequent appearance of cytoplasmic microtubules ending in the center of the bridge (Fig. 5). These microtubules, unambiguously identified in 10 of 21 diploid cases examined, emerge from the bridge along the normal (perpendicular) axis, and curve toward the bud if not initially directed within its margins. They frequently enter the bud itself, where their ends are generally ill-defined. Budding vesicles, 40 nm or more in diameter and of low electron density content, are invariably seen near the distal (bud) end of these microtubules. But the association may be fortuitous because the bud is generally filled with these vesicles.

**Stationary phase.** Yeast cells from cultures grown in YEPD to stationary phase are found predominantly to be unbudded single cells in G1 (20). We have examined both haploids and diploids under these conditions to determine the configuration of the spindle plaques with respect to this phase of the cell cycle. Although fixation for electron microscopy was not ideal, all observations demonstrated that these single cells possess a simple single plaque (Fig. 9). The half-bridge is often prominently stained as are the spindle microtubules; no satellites indicative of satellite-bearing single plaques are seen. The nucleus of a stationary phase cell frequently contains an aggregate of elongate structures similar to microtubules in form and dimensions. This aggregate differs from a spindle in that the apparent microtubules are of much greater electron density and lack any association with the spindle plaque. Similar aggregates have been described in stationary cells previously by Matile and his colleagues (8).

**Preparation for conjugation.** When two strains of opposite mating type are cultured together, the cells form mating pairs which undergo both cytoplasmic and nuclear fusion before the resulting diploid cells commence budding (13). Synchronization of the haploid cycles, facilitated by mating substances, appears to be required for conjugation, which occurs between unbudded cells arrested in G1 (4). To obtain haploid cells in preconjugatory arrest, we applied media enriched for the mating substances of α and α cells to haploid cultures of the opposite mating type. Electron microscopy (Fig. 10a) of arrested α cells (A364α) revealed that the nucleus invariably contains a plaque indistinguishable from the satellite-bearing single plaque formed prior to budding in growing cultures. Just as the double plaque is frequently directed toward the budding site in growing cultures, the satellite-bearing single plaque is usually found adjacent to the evagination prominent in α cells arrested by α hormone. This evagination contains a number of vesicles 40 nm in diameter and the distal ends of occasional extranuclear microtubules arising.

![Fig. 8. Frequency distribution of the angle at the bridge of the double plaque between the direction toward the neck of the early bud and the direction perpendicular to the plane of the bridge.](http://jb.asm.org/)
from the half-bridge of the satellite-bearing single plaque.

Cells of α mating type (S2072a) (18) remain isodiametric during inhibition of the cell cycle with a factor, lacking the evagination seen in α factor-arrested cells of a mating type. But they too bear the same sort of satellite-bearing single plaque seen in the first case (Fig. 10b).

Preconjugatory cells also accumulate in mating mixtures (described above) of these strains. The nuclei of these unbudded cells were found similarly to possess satellite-bearing single plaques.

**Conjugation.** After the accumulation of unbudded cells in mating mixtures, zygotes begin to appear. Fixation of these cultures and serial-

![Figure 9](http://jb.asm.org/)

**Fig. 9.** The nucleus (outlined by dots) of a diploid stationary cell showing a single spindle plaque (SP) and prominent half bridge (HBr) as well as dense fibers (DF) similar to microtubules. M, Mitochondrion.

![Figure 10](http://jb.asm.org/)

**Fig. 10.** Haploid cells arrested in the preconjugatory stable by mating substances. Both (a) the α mating type cell (strain A364A) arrested with a factor and (b) the α cell (S2072) arrested with a factor show satellite-bearing single plaques. S, Satellite; SP, spindle plaque.
section electron microscopy reveals various stages in the formation of zygotes, the fusion of the haploid nuclei, and the commencement of the budding cycle by the diploid zygote. These stages may be ordered into a sequence of events (Fig. 2g to l). Stable couples formed between the two haploid types before membrane fusion are not normally observed because our preparation involves wall removal, thereby separating the partners. The earliest paired stage readily found in our preparations has undergone partial perforation in the region of the fused walls (Fig. 2g). This results in the formation of a medial cytoplasmic channel similar to that demonstrated for Hansenula wingei by Conti and Brock (2).

No change in nuclear morphology from the state seen in preconjugatory stages is noted. As the 40-nm vesicles begin to fuse with the plasma membrane at the isthmus connecting the previous haploids, both nuclei retain satellite-bearing single plaques. These are always found adjacent to the isthmus, frequently residing on an evagination of the nuclear envelope oriented in this direction. Both plaques remain oriented toward the isthmus, and therefore toward one another through the passageway, as the nuclei move together (Fig. 11a). Cytoplasmic microtubules are frequently seen attached to the surface of the half-bridge. These microtubules often penetrate the isthmus and enter the cytoplasm of the opposite cell, where they may either bypass its nucleus or end in the vicinity of its plaque (Fig. 2h). Cytoplasmic microtubules appear to have some role in the interactions between the nuclei because stages are found in which they clearly interconnect the respective plaques of the two nuclei.

Other cases demonstrate that the nuclei then move together, led by their plaques, which meet one another near the isthmus. The narrow opening at this point apparently restricts the passage of much of the nuclear bulk except for the extended, plaque-bearing region. Frequent observations of binucleate zygotes with adjacent satellite-bearing single plaques on the two nuclei indicate a prolonged period in this state. Occasional cases of satellite orientation in antiparallel directions (Fig. 11b) are found; plaque reorientation to a parallel configuration (Fig. 11c) may be required at this stage. Formation of a single zygote nucleus then proceeds by the fusion of these two plaques, thereby resulting in fusion of the contiguous nuclear envelopes (Fig. 2i and j).

The mechanism of plaque fusion was examined in detail because our original observations (1) had suggested that the first double plaque of the zygote arose by end-to-end fusion between the half-bridges of the two satellite-bearing single plaques. Although the small size and contorted profiles of the fusion plaques in typical crosses defied structural interpretation, the larger size of plaques formed in crosses between diploid strains (2681-9A α/a X 1422-4C α/α) permitted us to interpret the mechanism of fusion from serial sections (Fig. 12). Rather than fusing end-to-end by their half-bridges, the satellite-bearing single plaques were found to join laterally along an edge parallel to the major axis of each plaque as diagrammed (Fig. 2h–j). Ordering events from several stages, we find that fusion occurs first between the two satellites and the two half-bridges. The plaques proper subsequently fuse directly together so the resulting fusion plaque retains the arrangement of a satellite-bearing single plaque along the axial dimension. In the lateral dimension, the fusion plaque is markedly curved because of the angle at which the parental plaques come together. This curvature often describes a hemicylindrical surface about an axis exterior to the nucleus and parallel to the axial dimension. Profiles in the lateral dimension are sometimes more V-shaped, each limb of the V being derived from one parental plaque. This is particularly evident in crosses between a haploid (A364Aa) and a diploid (1422-4C α/α) strain; here, the limbs of the V differ in length, reflecting the different widths of the parental plaques.

Formation of the fusion plaque is followed by plaque duplication as in a typical cell division cycle (Fig. 2k–l), the double plaque either retaining the curvature of the fusion plaque or becoming more nearly planar. The only obvious special feature is its location, which is within the isthmus produced by conjugation. Because plaque fusion frequently occurs immediately after perforation of the septum between the mating cells, the zygote nucleus is present before the isthmus is fully enlarged. This appears to prevent movement out of the narrow orifice by the nucleus and the double plaque arising on its surface.

The first bud of the zygote usually emerges from this same region, again revealing a spatial relationship between the double plaque and the budding site. The frequency of first buds arising in this medial location was 83, 74, and 42%, respectively, in crosses described earlier yielding diploid, triploid, and tetraploid zygotes. This decrease in the level of medial budding with increased ploidy is accompanied by an increase in the diameter of the isthmus. Suspecting that this may relieve the constraint on nuclear movement, we assayed nuclear position...
by Giemsa staining (12) and found nuclei within the isthmus less frequently among triploids and tetraploids. More importantly, we observed an invariant proximity between nuclei and buds: medial nuclei were found in zygotes with medial buds. Nonmedial nuclei were not only coincident with nonmedial buds, but always occurred at the same pole. Moreover, electron microscopy (Fig. 13) reveals that the double plaque lies near the base of the bud, which is entered by cytoplasmic microtubules extending from the bridge.

DISCUSSION

The observations reported in this paper define the manner in which spindle plaque devel-
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opment corresponds with other events of the cell division cycle and the conjugation process. Similar correspondence with cell cycle events had previously been found in the electron microscopy phenotypes (1) of cdc mutants. Taken together, their findings suggest not only that spindle plaque behavior is integrated with other cellular processes but also that the spindle plaques play a morphogenetic role in controlling these processes.

At the earliest stage of spindle plaque development in the cell division cycle, we find a single plaque. The same form is present during stationary phase, which represents an arrest of the cell cycle early in G₁ (5). In a later portion of the un budded phase of the cycle, the satellite-bearing single plaque arises. This stage is also achieved by cells undergoing conjugation. During zygote formation, spindle plaques of this form bear extranuclear microtubules associated first with the developing isthmus and later with the plaque of the other nucleus as the nuclei migrate together. The formation of a composite satellite-bearing single plaque, the fusion plaque, marks the end of the prolonged G₁ arrest coincident with conjugation.

The next stage of plaque development in the cycle is the double plaque, which appears to play a pivotal role in the behavior of both vegetative and zygotic cells. The present observations on vegetative cultures demonstrate that the double plaque is absent in all un budded cells and present in all budded ones. Moreover, plaque duplication and bud emergence are also interrelated spatially, as indicated by our measurements of the position of the double plaque and its preferred orientation toward the early bud. But this association does not of itself permit us to determine whether one of these events may have a role in controlling the other. Clues to their interactions, however, are found in the temperature-sensitive phenotypes of the cell division cycle mutants (1).

The lack of budding by cells mutant in cdc 28 is accompanied by the lack of plaque duplication; these strains retain the satellite-bearing single plaque characteristic of un budded cells both in the vegetative cycle and in preconjugatory arrest. The double plaque seen in early budding was found among these mutants only in cdc 4 strains, which show a unique capacity for repeated budding in the absence of nuclear division. It is, however, the phenotype of cdc 24 which provides perhaps the most compelling evidence that it is plaque duplication which is the independent event; the arrested cell undergoes complete cycles of plaque development and nuclear division in the absence of any budding, perhaps because of a specific inability to re-

Fig. 12. Serial sections of a fusion plaque in an early stage of nuclear fusion in a tetraploid (diploid × diploid) zygote. The curved spindle plaque (SP) is present in (a) and (b), and the composite satellite (S) is best seen in (c).
spond to the stimulus.

The distribution of extranuclear microtubules in vegetative cells suggests, moreover, that the double plaque controls bud emergence. These microtubules extend from the double plaque into the bud, their free ends lying near budding vesicles, which are thought to contain enzymes or precursors for the modification or growth of the bud wall (see reference 8). It seems likely that the microtubules transport these vesicles to the budding site, as in the cytoplasmic transport by microtubule systems in a wide variety of organisms (11).

This proposal predicts that the destruction of microtubules should impede bud emergence. S. cerevisiae is quite resistant to colchicine, but the derivative Colcemid has been shown to bind to a protein similar to other microtubule proteins (3). Although loss of microtubules has not been proven, application of this drug to cells emerging from stationary phase does indeed delay the appearance of the first bud, in accord with the hypothesis.

Such control of budding by the double plaque need not exclude other processes which restrict the location of budding. Although primulin staining reveals that the location of budding by diploid cells is relatively aspecific, haploid cells may bud at highly ordered adjacent loci (16). In other yeasts budding sites may be more precisely determined: the buds of Saccha-

romyces ludoigii emerge with previous bud scars (17) and those of Trigonopsis variabilis occur at the apices of the triangular cells (14). In such cases of specific bud localization, the proposed interaction of extranuclear microtubules may be restricted to a particular region of the cell cortex competent to respond to the stimulus.

Bud emergence in logarithmically growing cells is coincident not only with double plaque formation but also with chromosomal replication. Williamson (19) determined by autoradiography that the replication of nuclear deoxyribonucleic acid (DNA) begins approximately at the time of bud emergence and continues for 27% of the total cell cycle time. The data in this report demonstrate that the double plaque is present during this same period. Therefore, if these strains behave similarly, chromosomal DNA replication is coincident with the presence of the double plaque. Similarly, DNA replication in zygotes has been shown to be coincident with bud emergence (13), which we find to coincide in turn with duplication of the fusion plaque.

The cdc mutants reveal, however, that cells need not retain the double plaque until DNA synthesis is completed. Strains mutant in cdc 7, which fail even to initiate DNA replication (7), undergo plaque separation to form a complete spindle (2). The same spindle behavior is found
in mutants of cdc 8 and cdc 21 (1), which are defective in the elongation of replication of DNA. These strains become arrested with a complete spindle of length similar to that persisting during the period of bud enlargement. Whereas the spindles of normal vegetative cells again elongate rapidly as bud growth is completed, the spindles of these mutants do not undergo this final elongation. As in the earlier integration of plaque duplication with other early events of the cell cycle, an integrative mechanism appears to prevent this final elongation of the spindle if chromosome replication is not completed.

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LITERATURE CITED