

## A HIGHLY ORDERED RING OF MEMBRANE-ASSOCIATED FILAMENTS IN BUDDING YEAST

BRECK BYERS and LORETTA GOETSCH. From the Department of Genetics, University of Washington, Seattle, Washington 98195

The budding yeast, *Saccharomyces cerevisiae*, is a useful organism for cytological analysis of the cell division cycle not only because of the availability of numerous genetic variants (5), but also because of the comparative relationship of yeast with other eucaryotes. In addition to the persistence of the nuclear envelope throughout mitosis, the cell division cycle of *S. cerevisiae* differs from that of most other eucaryotes in the asymmetry of daughter cell formation, the growth of the yeast cell wall being confined principally to the emerging bud (9). After nuclear division in the narrow neck between the bud and the original cell, cytokinesis must then separate the daughter cells only at this limited connection. To determine the role of the neck in bud growth and in cytokinesis, we have examined this region by electron microscopy of serial thin sections. We find, as reported here, a highly ordered ring of 10-nm filaments lying along the interior surface of the plasma membrane.

### OBSERVATIONS

The sublamellar ring of 10-nm filaments in the neck of the bud has been detected in every strain of growing cells examined. The observations reported here were made on vegetative cultures of diploid strain AP-1 prepared for serial section electron microscopy by conventional methods, as described previously (1). Selecting representative stages of progress through the cell cycle from a fixed culture of these cells, we find that the filaments are undetectable in unbudded cells but are present throughout most of the budded phase. Their apparent absence before the bud appears is substantiated by the fact that the ring is still not well developed during the early phase of bud emergence (Fig. 1 *a* and *b*). At this stage, sections of the ring may contain fewer than 10 filaments, and these are often found to be discontinuous in serial sections.

As the bud enlarges, the ring undergoes further

development to a stable configuration with 20–30 filaments visible in section (Fig. 2). Serial reconstruction reveals that the ring now consists of a highly ordered monolayer of continuous filaments, each about 10 nm in diameter, spaced at intervals of 28 nm along that portion of plasma membrane which is curved inward. In transverse section (Fig. 2 *a* and *b*), each filament is attached to the inner surface of the plasma membrane along a connecting zone 5–10 nm wide. Lateral extensions about 3-nm thick appear to interconnect adjacent filaments at a distance of about 12 nm from the inner aspect of the unit membrane (Fig. 2 *b*). In grazing sections of the ring, longitudinal profiles of the filaments reveal an axial region of greater density (Fig. 2 *c* and *d*). This dense axial zone, occasionally appearing as a pair of fine lines 5 nm apart, is surrounded by a zone of intermediate density 8–12 nm wide. Faint striations cross the interspace at an angle of about 70° to the filaments; these may represent another view of the lateral extensions seen in transverse section.

Finding the filaments to be continuous in several complete sets of serial sections through the rings of well-oriented cells, we conclude that the filaments must be arranged around the neck either as closed circles or as the gyres of a helix. To determine the actual arrangement, we followed the paths of individual filaments by successively superimposing transparencies of micrographs from adjacent sections. Taking into account the orientation of sections and of micrographs prepared from them, we have defined the paths followed by the filaments in four rings displaying apparent continuity; these include one three-stranded negative helix and three positive helices with one, four, and five strands, respectively. We conclude that the filaments of the ring are usually arranged as helices in which both the handedness and number of strands are indeterminate.

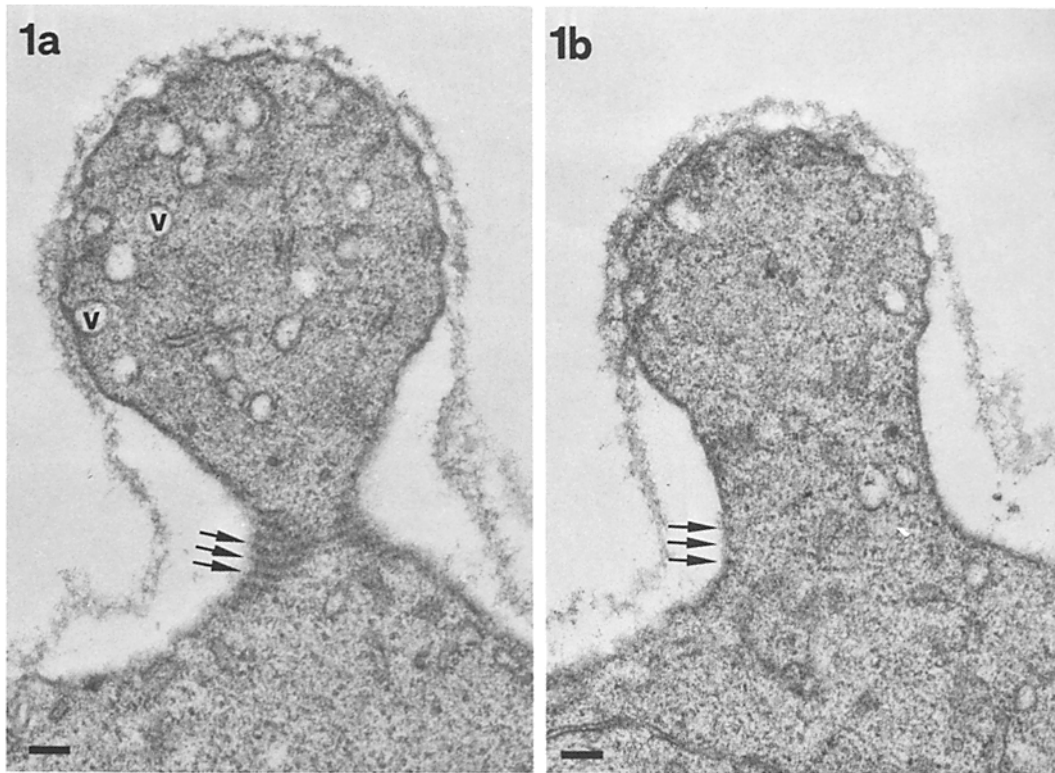


FIGURE 1 Sections from a serial set through an early bud laden with vesicles (v). Bud diameter to cell diameter, 1:9. (a) A grazing section of the ring reveals filaments (arrows) in longitudinal view. (b) A medial section shows them in transverse view. Bars are  $0.1 \mu\text{m}$ .  $\times 55,000$ .

The circumferential orientation of this array is analogous with that of the contractile ring of cleaving animal cells (12). To determine whether contraction occurs here as well, we measured the diameter of the ring in electron micrographs of cells from a growing culture. Employing bud size as an indicator of progress through the cell cycle (14), we found by a least squares approximation of data from 14 cells that the ring and neck increased in diameter by 29%, from  $0.42 \mu\text{m}$  to  $0.54 \mu\text{m}$ , during bud enlargement. Furthermore, observations of several cells with mature buds indicated that the ring never undergoes contraction but instead disappears at the time of cytokinesis, which is effected by vesicle fusion within the neck (Fig. 3). Among eight cases of cells fixed during this process, none display any remnants of the ring, nor is there any decrease in the overall diameter of the neck. The fusing vesicles instead form a septum consisting of two plasma membranes about 100 nm apart in a plane transverse to the axis of the filament-free neck. The irregular contour of the aperture remaining during closure further obviates

a contractile process, which would result in a more circular contour.

Attempts were also made to determine whether these 10-nm filaments might contain actin, as do the 6 nm "microfilaments" of the contractile ring (13). Treatment of growing yeast with cytochalasin B, which has been demonstrated to dissociate the microfilaments of the contractile ring (12), had no effect on growth unattributable to the dimethylsulfoxide added as solvent. Nor were we able to determine whether these filaments, like those known to contain actin, could be decorated by heavy meromyosin (7, 10, 13), because they were unstable to the prerequisite extraction with glycerol.

#### DISCUSSION

The filamentous ring described here occupies a location analogous with that of the "contractile ring" in cleaving animal cells (8, 12). Both are composed of circumferential filaments underlying the plasma membrane in the narrow region where cell separation will occur. There are, however,

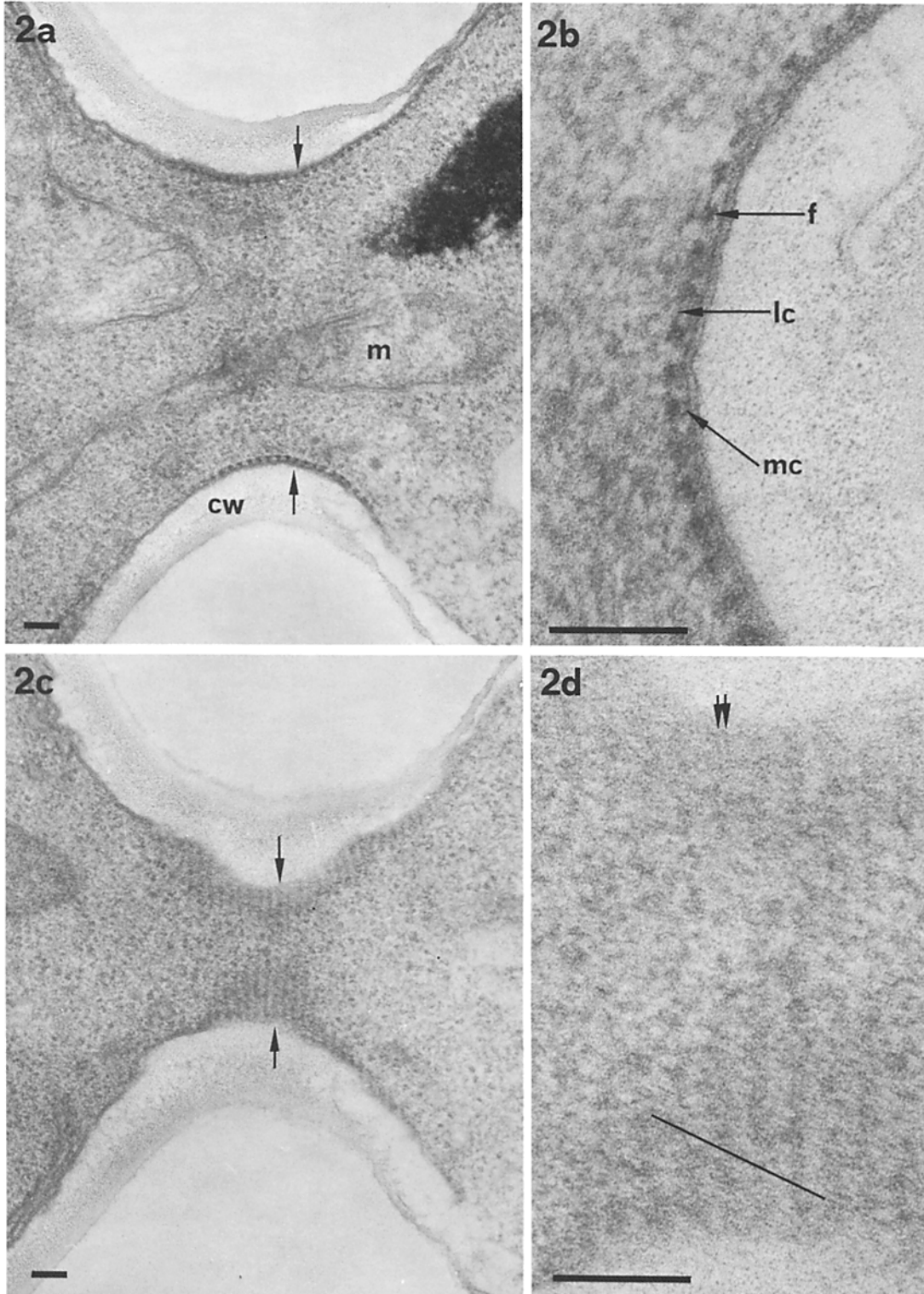


FIGURE 2 Sections from a serial set through a large bud. Bud diameter to cell diameter, 2:3. (a) Filaments are seen in transverse section along the inner surface of the plasma membrane. Arrows indicate profiles of a single filament followed through the serial sections. Mitochondrion (*m*); cell wall (*cw*).  $\times 55,000$ . (b) Higher magnification of the section adjacent to that in *a* shows lateral connections (*lc*) between filaments (*f*) as well as connections (*mc*) with the trilaminar plasma membrane.  $\times 220,000$ . (c) In a grazing section of the ring, arrows again indicate the same filament as in *a*.  $\times 55,000$ . (d) Higher magnification of a portion of *c* reveals faint diagonal striations (parallel with the line segment) and regions in which the medial dense region is double (double arrows). Bars are  $0.1 \mu\text{m}$ .  $\times 220,000$ .

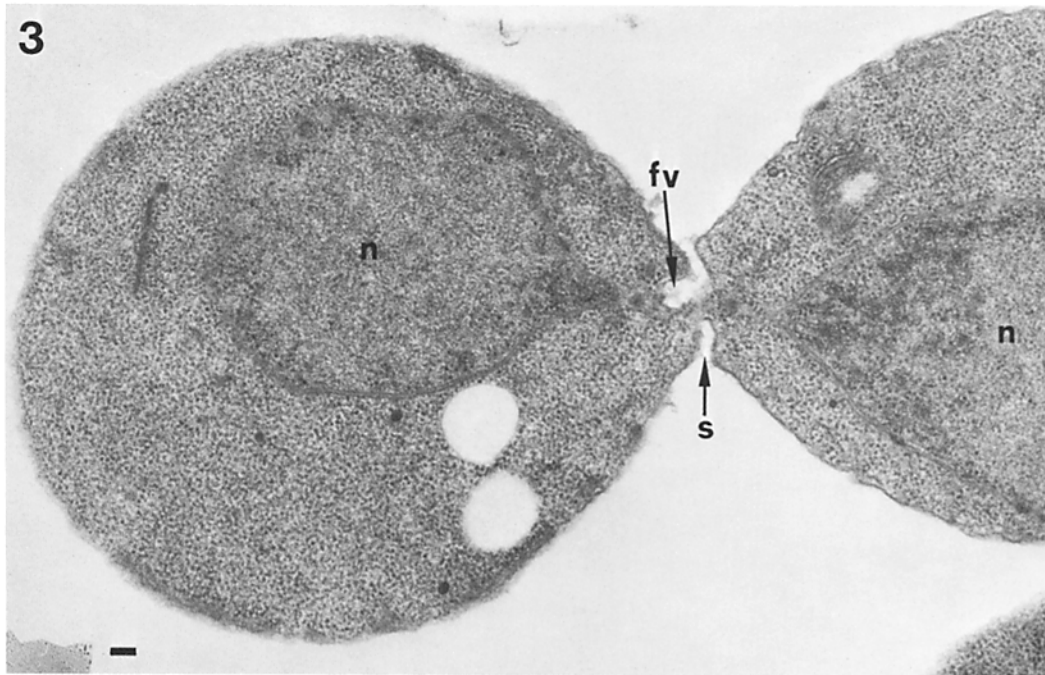


FIGURE 3 A cell undergoing cytokinesis by the fusion of vesicles (*fv*) to form a septum (*s*) at the middle of the filament-free neck. Nuclei (*n*). Bar is 0.1  $\mu\text{m}$ .  $\times 35,000$ .

striking differences between these structures. The filamentous ring of yeast arises during bud emergence and retains a constant diameter within the neck of the growing bud. The contractile ring, on the other hand, arises in coincidence with the cleavage furrow and undergoes active contraction during furrow constriction (12). Moreover, the filamentous ring of yeast is a highly ordered monolayer of 10-nm filaments (of unknown composition) in intimate association with the plasma membrane, whereas the actin-containing 6-nm filaments of the contractile ring of animal cells form a less highly ordered array extending more deeply into the cytoplasm (10, 12, 13).

The role of the filamentous ring in yeast may be indicated by its position within the neck of the bud. The margins of the ring coincide with the inwardly curved portion of the cell surface. Schroeder interprets an analogous distribution of the contractile ring in animal cells to indicate that the filaments counteract the normal outward curvature of the cell surface (12). A similar proposal may perhaps be inapplicable to yeast, in which the plasma membrane appears to be constrained by the overlying cell wall and no diminution of diameter occurs. On the other hand, constraint by the surrounding wall may be ineffective during bud emergence, when the wall of this region must lose

its rigidity in order to expand. The ring might then limit surface expansion to dimensions appropriate for budding.

Alternatively, the filamentous ring may play a role in the deposition of specific components in the overlying region of the cell wall. The bud scar, which occupies this site after cytokinesis, contains the majority of the chitin of the cell wall (2). Fluorescence microscopy of growing cells stained with a brightener indicates that the deposition of chitin in the circular rim of the bud scar is initiated early in bud emergence (6), when the ring first appears. During subsequent bud growth, the filamentous ring is suitably situated to interact with the complex chitin synthetase system proposed by Cabib and his colleagues (3).

Upon the completion of bud growth, cytokinesis is initiated within the neck by the fusion of vesicles with the plasma membrane, much as occurs in the phragmoplast during plant cytokinesis (11). The primary septum of the bud scar is generated by the deposition of chitin in the space between the pair of apposed membranes which are formed by vesicle fusion(3). The fact that the disappearance of the filamentous ring closely precedes septum formation suggests that these events may be functionally interrelated. One may propose, for example, that the filamentous ring inhibits vesi-

cle fusion until the appropriate phase of the cell division process. Perhaps the analysis of conditional mutants defective in cytokinesis (4) will clarify the role of this filamentous ring.

#### SUMMARY

In *Saccharomyces cerevisiae*, a highly ordered ring of 10-nm filaments is intimately associated with the plasma membrane within the neck of the bud. The ring is formed during early bud emergence and disappears when cytokinesis begins.

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