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Structural Changes in the Cell Wall of *Schizosaccharomyces pombe* During Cell Division

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ABSTRACT

STREIBLOVÁ, EVA (Czechoslovak Academy of Sciences, Prague, Czechoslovakia), I. MÁLEK, AND K. BERAN. Structural changes in the cell wall of *Schizosaccharomyces pombe* during cell division. J. Bacteriol. **91**:428–435. 1966.—Individual stages of growing and dividing cells of *Schizosaccharomyces pombe* were studied by means of fluorescence and electron microscopy with the use of metal-shadowed isolated walls, replicas, and ultrathin sections. Vegetative cells were found to contain division scars (six at the most); their formation and structure are described. More data on the growth of arthrospores were obtained. New structural observations were made on the architecture of the cell wall (original wall ring, polar cell wall, plug wall band, additional wall ring). Structural changes of cell surfaces and lateral walls during fission are represented schematically to the fourth generation. The question of origin of the septum is discussed, and on this basis the entire structure of the cell wall is interpreted.

The architecture of the cell wall of *Schizosaccharomyces* is best understood if the individual stages of growing and dividing vegetative cells are examined.

The cell wall of this group of yeasts has never been investigated independently. Light microscopy yielded some principal data on fission, in particular on the formation of a transverse septum (8, 11, 16). Cell growth was studied by use of fluorescent antibodies (14).

Very little is known about the ultrastructure of the cell wall. It has been examined only in ultrathin sections (5, 13). Its relatively low electron density makes it difficult to detect layers and other particular structures in electron micrographs. Electron cytochemistry of ultrathin sections of dividing yeast cells (18) confirmed the results of chemical analysis of their cell wall (7, 12, 15).

A new staining method for yeast cell walls with the use of the fluorochrome primulin (23, 24) was employed. Individual cells of *S. pombe* were found to contain a greater number of so-called division scars. It was assumed that a cytokinetic process is accompanied by structural changes in lateral cell walls that will be amenable to investigation for a number of generations.

Fluorescence microscopy which makes it possible to follow readily a greater number of cells at various stages of division was combined with electron-optical examination of cell surfaces, metal-shadowed isolated cell walls, and ultrathin sections. This approach enabled us to study the cell wall during almost all stages of cell division on a submicroscopic level.

MATERIALS AND METHODS

Culture and cultivation. S. pombe 221 from the National Collection of the Czechoslovak Academy of Sciences was grown in the synthetic medium of Olson and Johnson (20) containing: glucose, 10 g; $NH_4H_2PO_4$, 6 g; KH_2PO_4, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; sodium citrate, 1 g; L-asparagine, 2.5 g; biotin, 20 μg; calcium pantothenate, 0.5 mg; inositol, 10 mg; thiamine, 4 mg; pyridoxine, 1 mg; zinc (as sulfate), 400 μg; iron (as ferrous ammonium sulfate), 150 μg; copper (as sulfate), 25 μg; and distilled water to 1 liter. The pH was adjusted to 5.0 with phosphoric acid. The culture was grown at 28 C for 24 to 28 hr on a rotary shaker.

Fluorescence microscopy. Methods of staining preparations and data on the fluorescence microscope were presented previously (23, 24, 26).

Electron microscopy. Washed cells were mechanically crushed with Ballotini beads. The cell-wall fraction was obtained by centrifuging the crushed cells in physiological saline three times and washing them

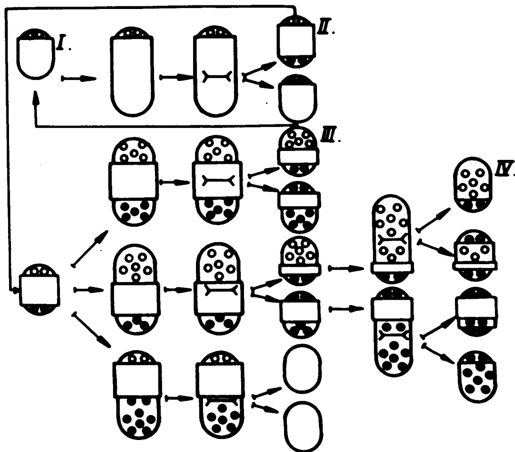

FIG. 1. *Schematic representation of fission in Schizosaccharomyces pombe (partially up to the fourth generation). (I) Cells with a single scar are doubled by longitudinal growth and a septum is formed. (II) Cleavage of the septum gives rise to two cells; the single-scar one undergoes the same process as the original cell, whereas three alternatives of terminal growth exist for a two-scar cell. (III) Cells of the third generation have two scars; the original wall ring has a different width and localization. The third alternative of the terminal growth has not as yet been sufficiently studied, and therefore the cells of the third generation are drafted without any structures. (IV) After growth of the arthrospore from one pole, the fourth generation contains three-scar cells in addition to single-scar ones. The original wall ring is shown as white area; scar plugs, polar cell walls, and additional wall rings (see Fig. 2) by small circles.*

three to five times with water until the purity required for observation was attained.

Metallized preparations were obtained by placing clean walls onto supporting Formvar films and shadowing with carbon and platinum at a 30° angle (22).

For making ultrathin sections, the isolated walls were fixed at 4 C for 2 hr in 2% $KMnO_4$ and in 1% OsO_4 (0.14 M sodium acetate-Veronal buffer, *p*H 4.5). After being washed three times with water, the walls were embedded in 2% agar at 40 C. After dehydration in an ascending acetone series, the objects were embedded in Vestopal W. Polymerization took place at 60 C and lasted for 24 hr. Sections were cut with a Porter-Blum microtome equipped with a glass knife.

Carbon replicas were obtained as described previously (4, 26).

RESULTS AND DISCUSSION

The vegetative cell of *S. pombe* is usually cylindrical with hemispherical ends. The most distinct structures on the cell wall surface are division scars. The division scars represent permanent changes of the cell wall, formed at the point of cleavage of the septum. The term division scar was coined by Mitchison (16).

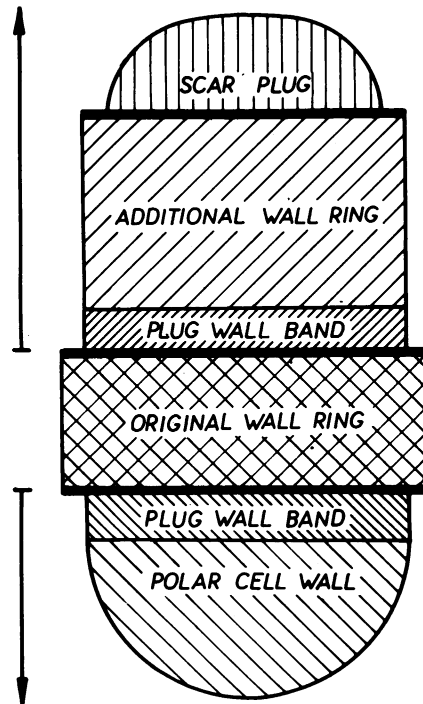

FIG. 2. *Diagram of a three-scar cell. Arrows indicate the direction of the terminal growth (terminal growth continues on the lower pole). The scar plug is the region of cell wall on the cell pole bounded by the scar margin. This region has the same properties as the plug wall band. The original wall ring is the oldest part of the cell wall; it is bounded on both sides by scar margins. The plug wall band is a belt of the scar plug wall; it preserves the properties of the scar plug and is a part of the polar cell wall and of the additional wall ring. The polar cell wall is a region of the younger cell wall of the cylindrical cell pole; it is bounded by one scar margin, and has a plug wall band determining the direction of wall extension. The additional wall ring of the younger wall, bounded on both sides by scar margins, has a plug wall band determining the direction of wall extension.*

Vegetative reproduction of *S. pombe* results in the formation of cells containing different numbers of division scars (Fig. 1). In the first generation, two cells are formed after division of a single-scar arthrospore. A single-scar cell undergoes the same process of division as the original cell. A cell with two terminal scars can grow longitudinally in three ways: (i) equal segments of a new cell wall grow at both poles; (ii) newly synthesized sections are of unequal length; or (iii) the arthrospore grows from one of both poles. The last kind of terminal growth has not as yet been studied in detail by us. The cell wall of the original cell forms a ring at the extending arthrospore. Since the cell divides always in the

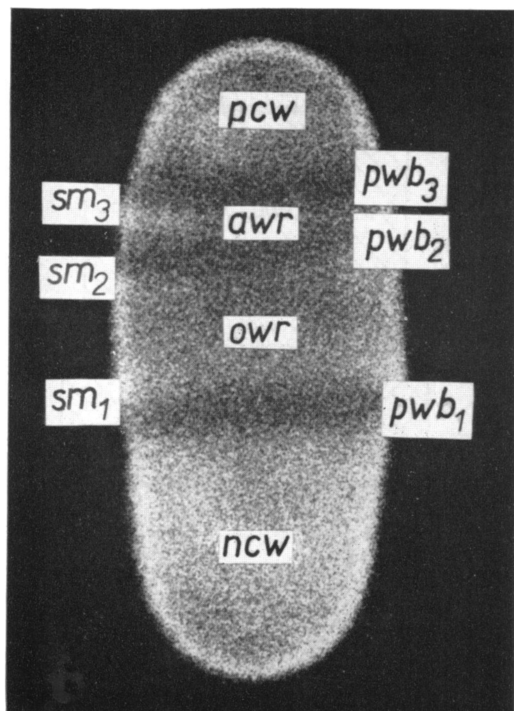

FIG. 3a. *Fluorescence photomicrograph of Schizosaccharomyces pombe cell with three division scars (pcw and ncw, polar cell walls; pwb_{1-3}, plug wall bands; sm_{1-3}, scar margins; owr, original wall ring; awr, additional wall ring).* × *3,000.*

middle, the septum is formed in the center of the original wall (Fig. 3b) or eccentrically (Fig. 3c), depending on the type of terminal growth. In the third generation, cells with two scars are formed in this way but the width of the original wall depends on the type of terminal growth. Cells with three scars are formed in the fourth generation. The scheme does not indicate the proportional representation of cells with different scar numbers, since the statistical evaluation of population according to the number of scars will be published elsewhere.

During cell division, observations were made on the structure of the cell wall. The principle of the cell wall organization follows from the architecture of three-scar cells (Fig. 2).

The margin of the division scar (sm) is formed by an annular thickening of the wall at the point of septum cleavage. Secondary fluorescence of the margin is of medium intensity (Fig. 3a, sm_{1-3}), about equal to that of the margin of a multiple scar (26). The margin of the bud scar of budding yeast cells fluoresces more intensively (25). Electron micrographs exhibit a distinct margin of the scar both in the metallized walls (Fig. 5, sm_{1-3}) and on the replicas where it resembles a protruding ridge (Fig. 6, sm_{1-3}). It follows from the ultrathin sections that the margin of a division scar which protrudes from the cell profile is formed by the entire original cell wall (Fig. 4a, sm).

The plug of a division scar (sp) is the area of

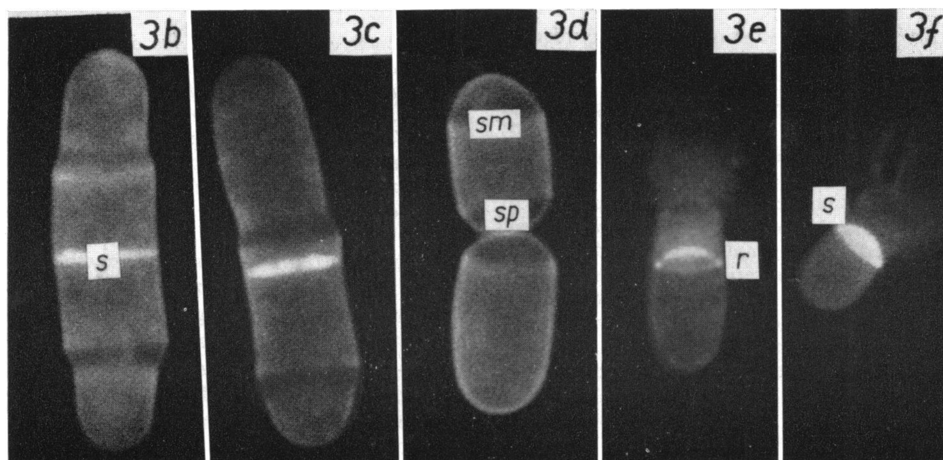

FIG. 3b–f. *Fluorescence photomicrographs of Schizosaccharomyces pombe in different stages of cell division. (b) Second-generation cell (see Fig. 1) with two scars; the septum (s) is in the center of the original wall ring.* × *3,000. (c) Second-generation cell (see Fig. 1) with two scars. The septum lies eccentrically in the original wall ring.* × *3,000. (d) Second-generation of cells immediately after septum cleavage (sm, scar margin; sp, scar plug).* × *3,000. (e) First phase of septum formation (r, ring).* × *2,500. (f) Terminated synthesis of the septum (s).* × *2,500.*

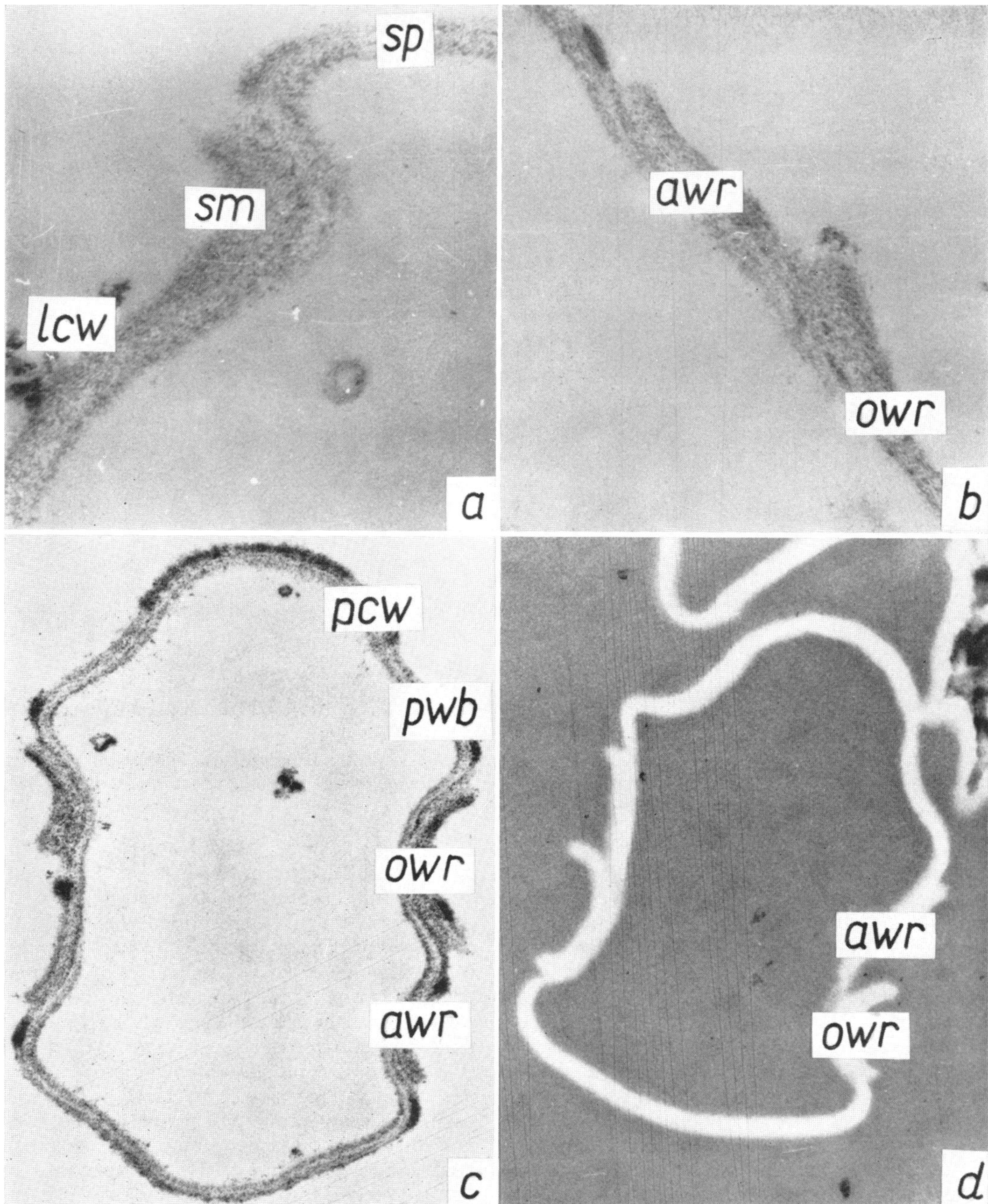

FIG. 4. *Ultrathin sections through a cell wall of Schizosaccharomyces pombe. (a) Longitudinal section through a division scar (lcw, lateral cell wall; sm, scar margin; sp, scar plug). Boundary between the scar margin and the plug is well marked. Fixed with $KMnO_4$. × 44,000. (b) Section through a part of the lateral wall (owr, original wall ring partially visible; awr, additional wall ring). Fixed with $KMnO_4$. × 44,000. (c) Longitudinal section through an isolated wall of a three-scar cell (pcw, polar cell wall; pwb, plug wall band; owr, original wall ring; awr, additional wall ring). Fixed with $KMnO_4$. × 22,000. (d) Longitudinal section through an isolated cell wall of a three-scar cell (awr, additional wall ring; owr, original wall ring). Fixed with OsO_4. × 22,000.*

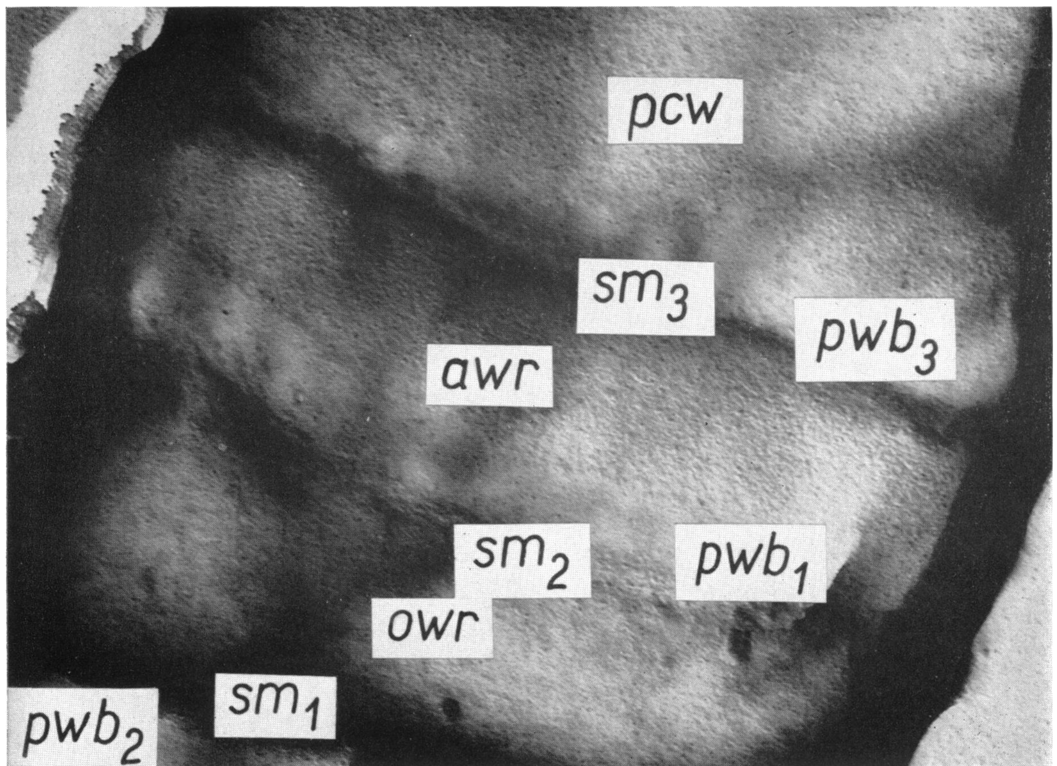

FIG. 5. *Isolated wall of a three-scar cell of Schizosaccharomyces pombe shadowed with carbon and platinum. View of a part of the surface [pcw, polar cell wall; sm_{1-3}, scar margins; pwb_{1-3}, plug wall bands (pwb_2 only partially visible); awr, additional wall ring; owr, original wall ring].* × *35,000.*

the cell surface that is bounded by the scar margin. It takes up the entire cell apex of a cylindrical cell and is slightly convex because of turgor. Like the plug of a bud scar and of a multiple scar (25), it shows negligible fluorescence (Fig. 3d, sp). The chemical composition of the three types of scar plugs is different (17, 18). In the isolated cell wall the plug has a different density. The structural material of the plug is attached to the internal surface of the original cell wall so that there is no continuity between the lateral cell wall and the plug wall (Fig. 4a, sp).

The cells with two or more scars possess an oldest original wall ring (owr). Its width and location depend on the number of divisions which the cell has gone through and on the manner of the terminal growth of the arthrospore. The original wall ring (Fig. 3a, 4b, c, d, 5, 6; owr) is bounded on both sides by scar margins. The new cell wall extends, while growing, centrifugally from this ring to one or both sides (Fig. 2). In ultrathin sections, the original wall ring forms an independent layer (Fig. 4b, c, d; owr), which is being pushed above the surface by walls forming from the scar plugs.

Fluorescent antibodies showed that the sites of cell wall extension are the cell apices bounded by division scars (14). The scars can serve as markers in studies of wall extension during cell growth. The new cell wall (pcw) shows, in metal-shadowed specimens (Fig. 5, pcw), in replicas (Fig. 6, pcw_{1-2}), in ultrathin sections (Fig. 4c, pcw), and in fluorescence microscopy (Fig. 3a, pcw), the same properties as the original wall ring. It grows on one or both poles again to double the length of the original arthrospore.

More detailed investigation reveals that the cell wall, possessing the same properties as the original one, is synthesized from the scar plug only at a certain distance from the scar margin. Between the scar margin and the newly synthesized wall, there remains a belt that we designated as plug wall band (pwb) as it preserves the properties of a scar plug. It borders on the scar margin always in the direction of growth of a new wall (Fig. 2). Secondary fluorescence of this

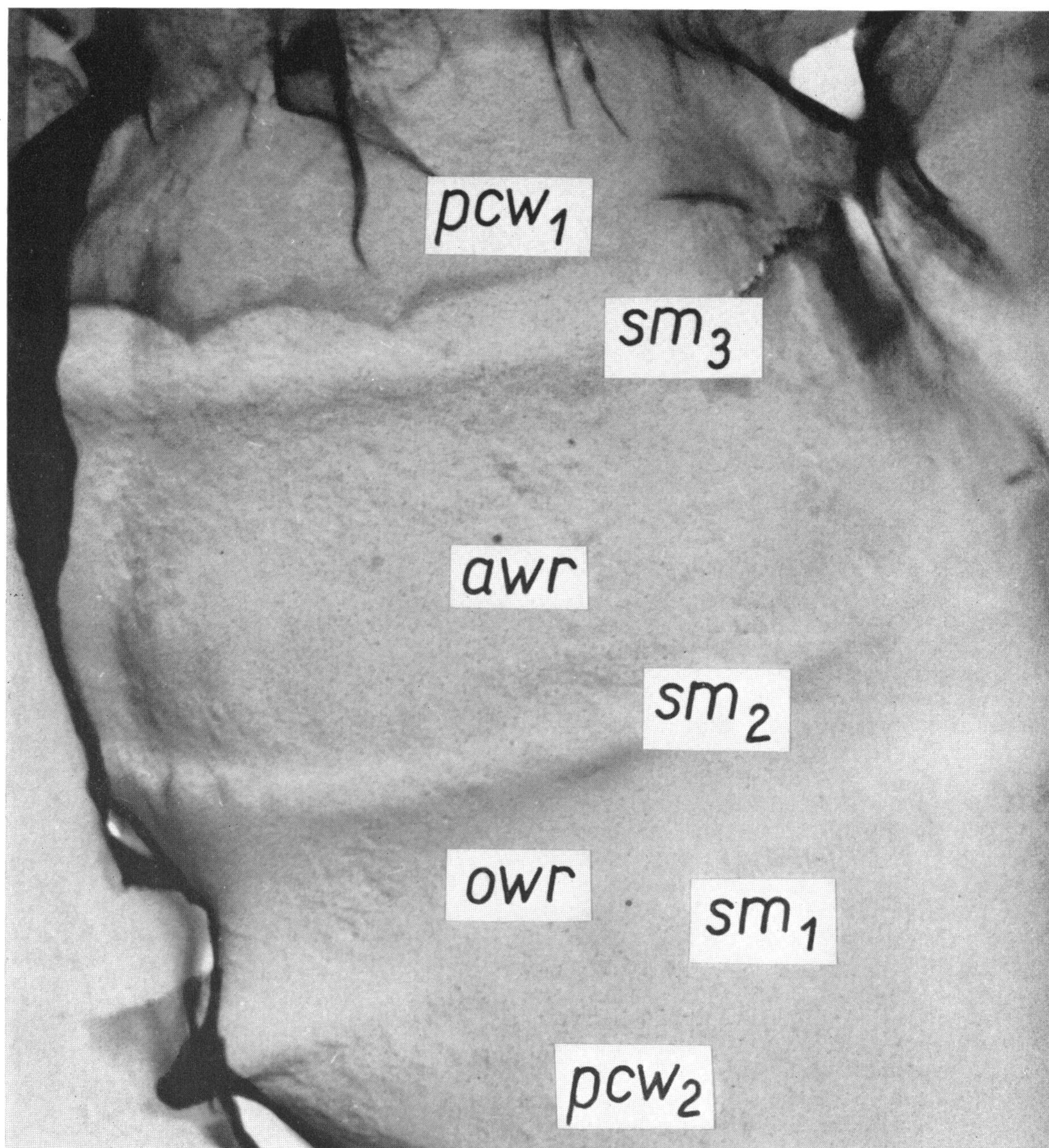

FIG. 6. *Carbon replica of a three-scar cell of Schizosaccharomyces pombe. View of a part of the surface* ($pcw_{1,2}$, *polar cell walls; awr, additional wall ring; owr, original wall ring;* sm_{1-3}, *scar margins). The plug wall bands are not visible.* × *28,000.*

zone is negligible (Fig. 3a; pwb_{1-3}). On the cell wall surface, the plug wall forms a belt with lower granularity (Fig. 5, pwb_{1-3}). In ultrathin sections fixed with $KMnO_4$, the zone can be defined from the concentration of electron-opaque material at the outer margin of the growing scar plug (Fig. 4c, pwb).

The vegetative cell grows only longitudinally, the increase in volume being proportional to the increment in length. As soon as it is approximately doubled, a transverse septum begins to form by centripetal growth without cell plate (5, 11). The cytoplasmic membrane accompanies closely the growing wall septum (11). Fluorescence microscopy is a method which makes it possible to follow readily the formation of the septum. Primulin fluorescence reveals the annular growth of the septum. During the first stage, a

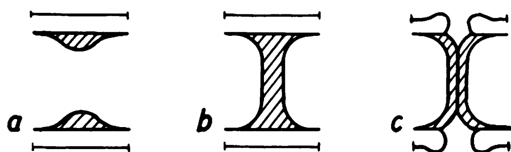

FIG. 7. *Diagrams of division scar formation. Hypothetical longitudinal section through the cell wall (hatching shows the cell wall synthesized de novo). (a) The septum is founded as a ring adjacent to the lateral cell wall. (b) Centripetal growth of the ring gives rise to a transverse septum. (c) During cleavage of the septum, division scars are formed—the scar plug from the septum material, margin of the scar from lateral cell wall.*

yellow-green ring is formed (Fig. 3e, r), fluorescence increases, and the central opening gradually closes. After termination of synthesis of the septum, its entire area has approximately the same fluorescence as the margin of a bud scar (Fig. 3f, s).

Fluorescence microscopy permits one to follow directly the process of scar formation. The septum splits, its fluorescence rapidly decreases, and the cells are pushed apart by convex scar plugs. At the same time, both cells display slightly fluorescent scar margins at the location of the former septum (Fig. 3d). In ultrathin sections, a boundary between the scar plug and scar margin is apparent (Fig. 4a), which indicates the independent origin of both structures. It is deduced from this finding that the septum is synthesized independently. Graphically, it is possible to demonstrate the formation of division scar in three phases (Fig. 7). Indications of a boundary between the septum and the lateral wall are apparent in Maclean's work (13). Our findings are at variance with those of Conti and Naylor (11), who based their deductions on ultrathin sections and explained the formation of the septum by the growth of the internal part of the lateral cell wall. In this case, the plug of the division scars would be formed by the above-mentioned internal layer of the cell wall and the margin by the remaining external part.

In cells from three scars upward so-called rings of additional wall (awr) form on the cell wall. These rings are the younger parts of the cell wall, bounded by scars on both sides (Fig. 3a, 4b, c, d, 5, 6; awr). In three-scar cells, the outer scar forms during the next division. As distinguished from the original wall ring, the additional wall ring has always a plug wall band, indicating the direction of the wall growth. Depending on the number of divisions, there are further additional wall rings, the formation of which has been studied.

Maclean (13) stated that the cell wall thickness of *S. pombe* varies from one part of the cell to another and that old division scars may result in areas of greater thickness. However, like Conti and Naylor (5), he did not obtain any structural details of the cell wall in ultrathin sections. In contrast to the authors mentioned above, we used isolated cell walls. After fixation with $KMnO_4$ (Fig. 4a, b, c), the boundaries between individual zones were well contrasted, whereas fixation with OsO_4 (Fig. 4d) yielded contours of walls in the embedding medium without contrasting inner structure. Both types of fixation produced mutually supplementary results concerning the structure of lateral walls.

Our concept of cell-wall architecture in dividing yeast cells is based on the assumption that during fission the septum is formed de novo by centripetal growth. In plant cells where the transverse septum is formed with the aid of the cell plate during cell division, new cell wall is also formed (6). Since, during terminal growth of dividing yeast cells, the new wall is synthesized from the material of the septum, vegetative cells contain rings of different ages bounded by scars. Their architecture differs from that of the cell wall of budding yeasts where longitudinal layers have been studied (1, 2, 3, 9, 10, 17, 19, 21, 27, 28). Since our concept of cell wall structure is based on cytological observations, it will be necessary to establish a correlation between the wall structures formed and metabolic processes by means of incorporation of radioactive substances into the individual rings.

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