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Cell Division in Yeasts: Movement of Organelles Associated with Cell Plate Growth of *Schizosaccharomyces pombe*[1]

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Electron microscopy of dividing fission yeast cells shows establishment of an annular rudiment (AR) of electron-transparent material under the old cell wall as the first sign of elaboration of the cell plate. The AR grows centripetally, finally closing at the mid-point of the cell. During the inward growth of the AR it is thickened by addition of denser material which becomes the scar plug after fission; the electron-transparent material is lost at fission. Lying always between the cytoplasmic membrane and the cell wall is a dark layer of variable thickness. This layer becomes markedly thickened into a fillet at the base of the centripetally growing cell plate. The fission process begins after the cell plate is completely elaborated. One striking feature of fission is the migration of dense material from the fillet at the base of the cell plate outwardly through the matrix of the cell wall to its final resting place as a dark ring, a "fuscannel," adjacent to the fission scar. The inclusion of Golgi bodies in many sections suggests their involvement in cell plate elaboration, presumably through production of the dense bodies which are seen to fuse with the dark layer proximal to the growing cell plate.

Consequent to the classical analysis of Mitchison (15), the fission yeast *Schizosaccharomyces pombe* has become widely used for cell cycle studies. Interestingly, our knowledge of its cell division processes is rather sparse. Nuclear events at mitosis are now well described (14), but the analysis of cytokinesis (fission in this case) is incomplete. For example, an interesting and heuristically useful study (24) emphasized the final product, whereas others (19) studied mostly the early stages of formation of the cell plate.

Our intention is to blend some older observations of fission (12, 15, 16, 19, 24) with new data, to attempt a more complete description. For instance, an "electron-dense ring" (24), hereafter called "fuscannel" (fuscus, L., being dark; anellus, L., small ring, diminutive of anus) by us, external to the cell wall at the division scar (15, 16), will be seen to derive from "materiel triangulaire dense" (19) found within the structure of the wall itself. However, that materiel triangulaire dense does not appear de novo within the wall but is clearly derived from a "dark layer of variable thickness" (12) which lies between the wall and the cytoplasmic membrane. Finally, we find reason to suggest a cytoplasmic origin for the substance of the dark layer.

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MATERIALS AND METHODS

Cloned cells of *S. pombe* NCYC 132, a haploid homothallic strain (3), were grown in 140 ml of malt extract broth (16; Oxoid; 2% wt/wt). The cultures were inoculated from a culture which had just reached stationary phase and shaken at 150 rpm at 32 C until harvested 3.5 h later.

The cells were harvested by low-speed centrifugation and washed twice in distilled water prior to fixation. Fixation of the yeast was done in freshly prepared 2% $KMnO_4$ for 30 to 60 min at room temperature. Fixed cells were dehydrated in acetone and embedded in Epon resin (11). Thin sections were stained in lead citrate as described by Reynolds (20). Electron micrographs were taken with a Philips EM 200 at 60 kV.

RESULTS

The most prominent cytoplasmic organelles in nondividing log-phase fission yeast cells are the electron-transparent vacuoles and the electron-dense vacuole-associated organelles (Fig. 1A; 2A). Mitochondria are plentiful and are generally distributed. Golgi bodies had not usually been found in nondividing fission yeast cells (6, 17) at the time this work was in progress, but are obvious in this study and have recently been discussed (10, 22). Scattered apparently at random are small (approximately 23–106 nm in diameter) electron-dense bodies comparable in density to the dark layer of variable thickness (Fig. 1A; 2A) which lies between the cytoplasmic membrane and the cell wall (12). The protrusions of this layer into the cytoplasm are clearly similar to those seen in *Saccharomyces cerevisiae* (5). Just inside the cytoplasmic membrane and parallel to it is the endoplasmic reticulum.

During cell division the vacuoles and vacuole-associated organelles appear to diminish. Most sections contain a recognizable vacuolar vestige which often lies near a Golgi body (Fig. 4D). The Golgi apparatus is prominent, with easily recognized profiles in many cells (Fig. 2A, B; 3F; 4A, B, D). (Figures 2A and 3F, with three Golgi bodies each, probably exaggerate the frequency, but are of the proper order.) It is obvious that the electron density of the Golgi apparatus is quite comparable with the densities of the small electron-dense bodies and the dark extra-cytoplasmic layer. Occasionally one sees the apparent fusion by reverse pinocytosis of small bodies with the dark extra-cytoplasmic layer (Fig. 1E; 3C, D, F; 4C, D).

The cell plate first appears as an electron-transparent annular rudiment (AR; Fig. 1C, 2B) lying in intimate contact with the innermost part of the cell wall (Fig. 1C, D; 2B; 3A, B, C). It seems at first glance to be a centripetally growing continuation of that innermost part of the cell wall, and has been reported as such (4). However, the AR has, on the average, much less electron density than the wall material to which it is apposed, and its affinity for primuline, a

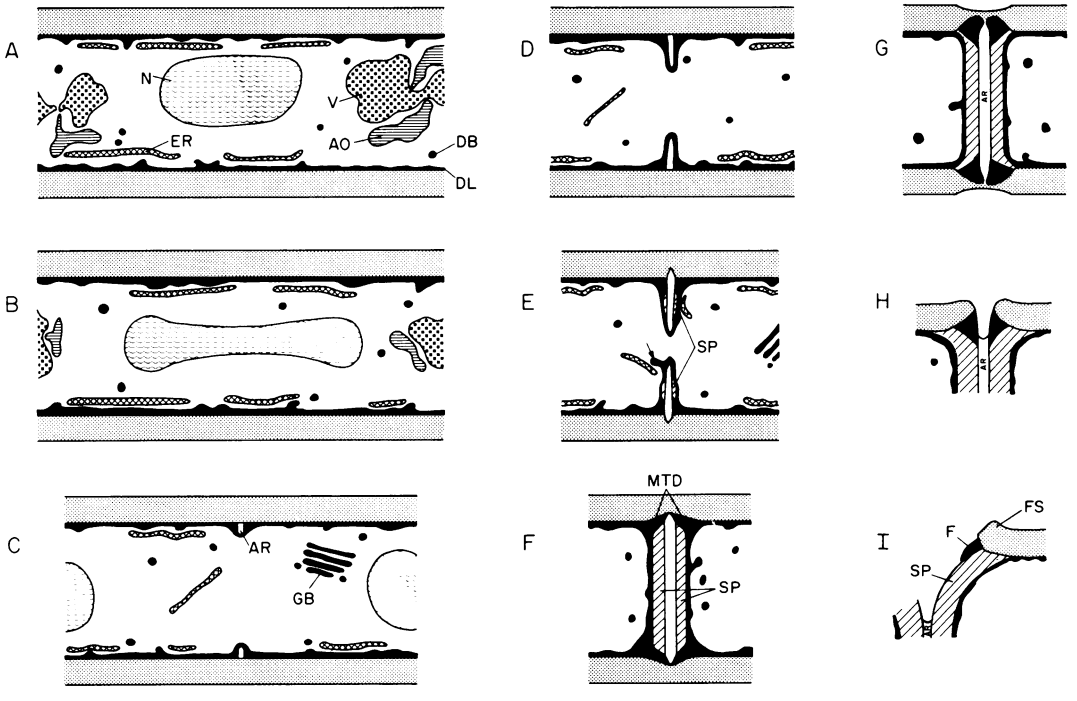

FIG. 1. *Fission of S. pombe. A–C, Mitosis and initiation of annular rudiment (AR). D, Establishment of dense fillets at base of AR. E, Initiation of scar plug layer (SP[24]); fusion of dense body (DB) with dark layer (DL, arrow). F, Migration of dense fillet into wall to become materiel triangulaire dense (MTD [19]); closure of pore. G, Growth of SP layer to make contact with old cell wall; initiation of fission by erosion of old cell wall. H, Outward movement of MTD to become fuscannel (F); fission scar (FS) ridge becomes apparent; AR material is lost at edge of layer. I, Fission almost complete; F at final disposition; SP layer new end of daughter cell; AR material nearly all lost.*

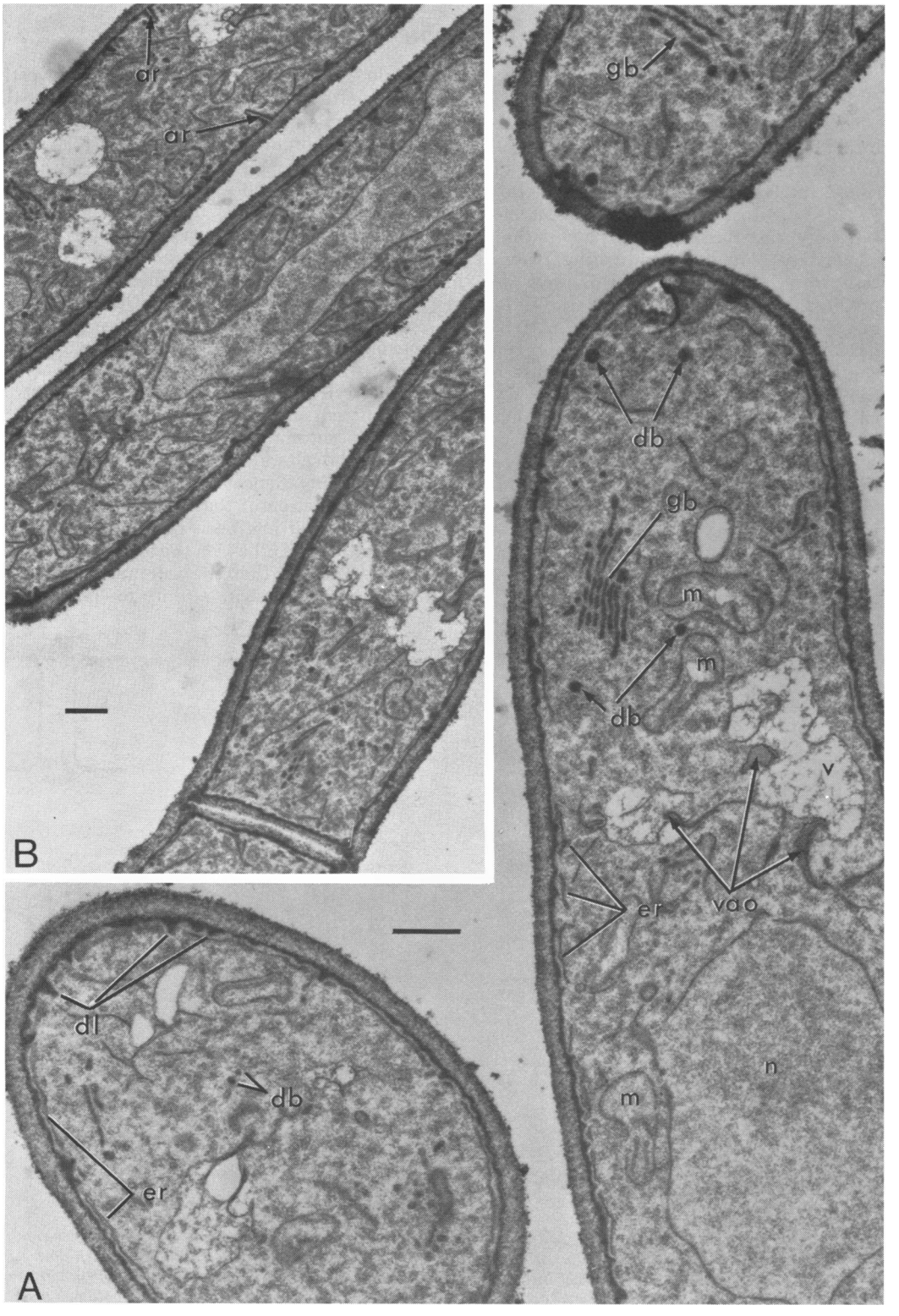

FIG. 2. *A, Normal premitotic cytoplasm; all magnification bars equal 0.5 μm. B, First signs of annular rudiment (ar) in postmitotic cell, shown just above a mitotic cell. Abbreviations: ar, annular rudiment; db, dense body; dl, dark layer; er, endoplasmic reticulum; gb, Golgi body; m, mitochondrion; n, nucleus; v, vacuole; vao, vacuole-associated organelle.*

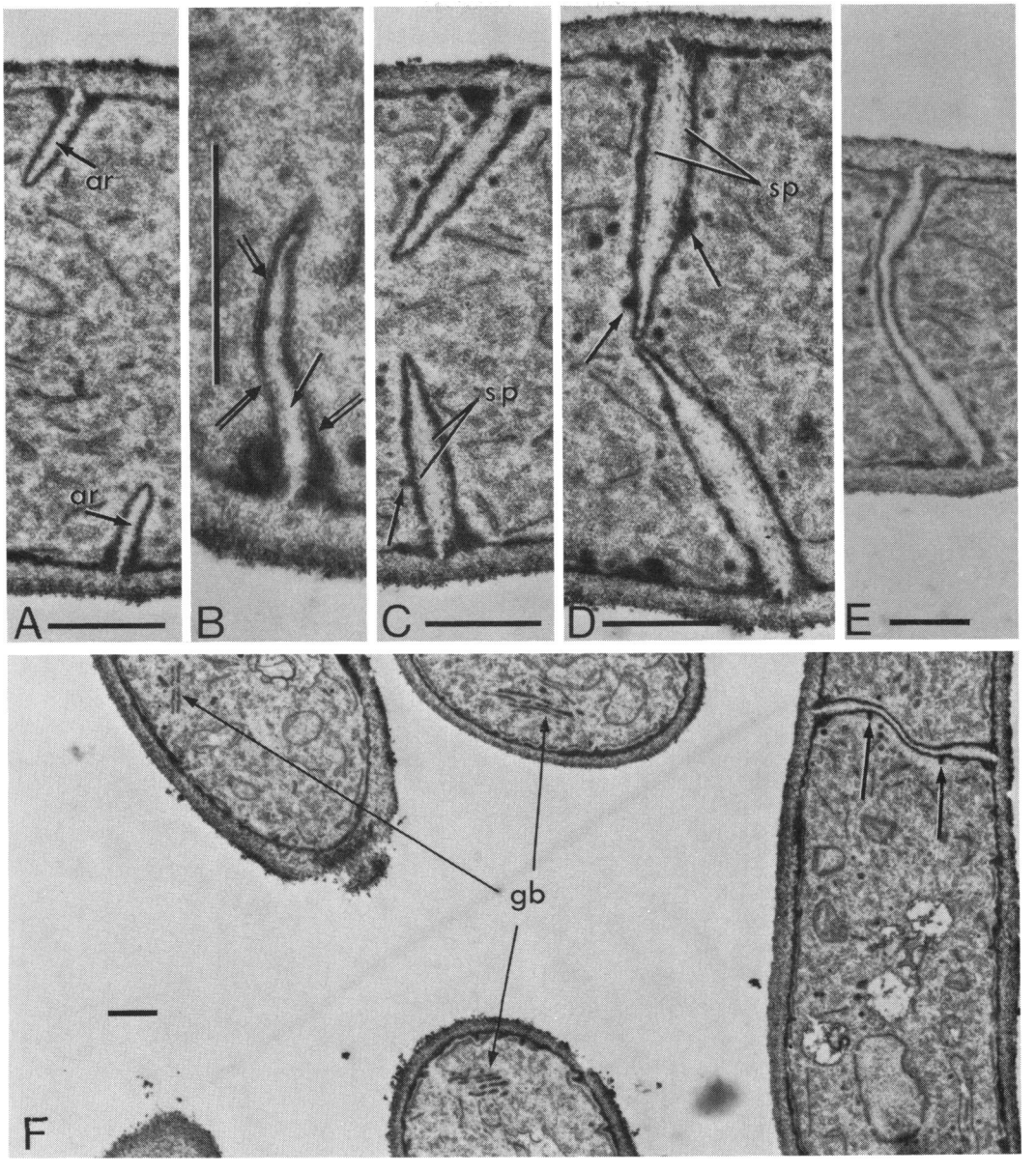

FIG. 3. *A, Centripetal growth of annular rudiment (ar) layer with thickening of dark layer to form fillets at base of ar layer; all magnification bars equal 0.5 μm. B, Highly magnified view of one-half cell plate, showing ar layer (single-shafted arrow), dark layer, and fillet covered entirely by cytoplasmic membrane (double-shafted arrow). C, Initiation of scar plug (sp) layer, and fusion of dense body with dark layer (arrow). D, Closing the pore; fusion of dense body with dark layer (arrows). E, F, Closure of cell plate; note Golgi bodies in neighboring cells; fusion of dense body with dark layer (arrows).*

fluorescing dye, is much greater than the rest of the wall (24, and unpublished data of our own), hence it differs in some manner from the old cell wall. The AR is ordinarily thickest where it touches the old cell wall and seems to taper toward its centripetal edge.

The growing AR of the cell plate is covered (Fig. 2B; 3A, B) with the dark layer which lines the rest of the cell wall and which lies outside the cytoplasmic membrane; hence, synthesis of the cell plate occurs remotely from the cytoplasm. The dark layer is markedly thicker, like a "fillet" (i.e., deltoid in cross section) at the base of the rudiment, and it becomes progres-

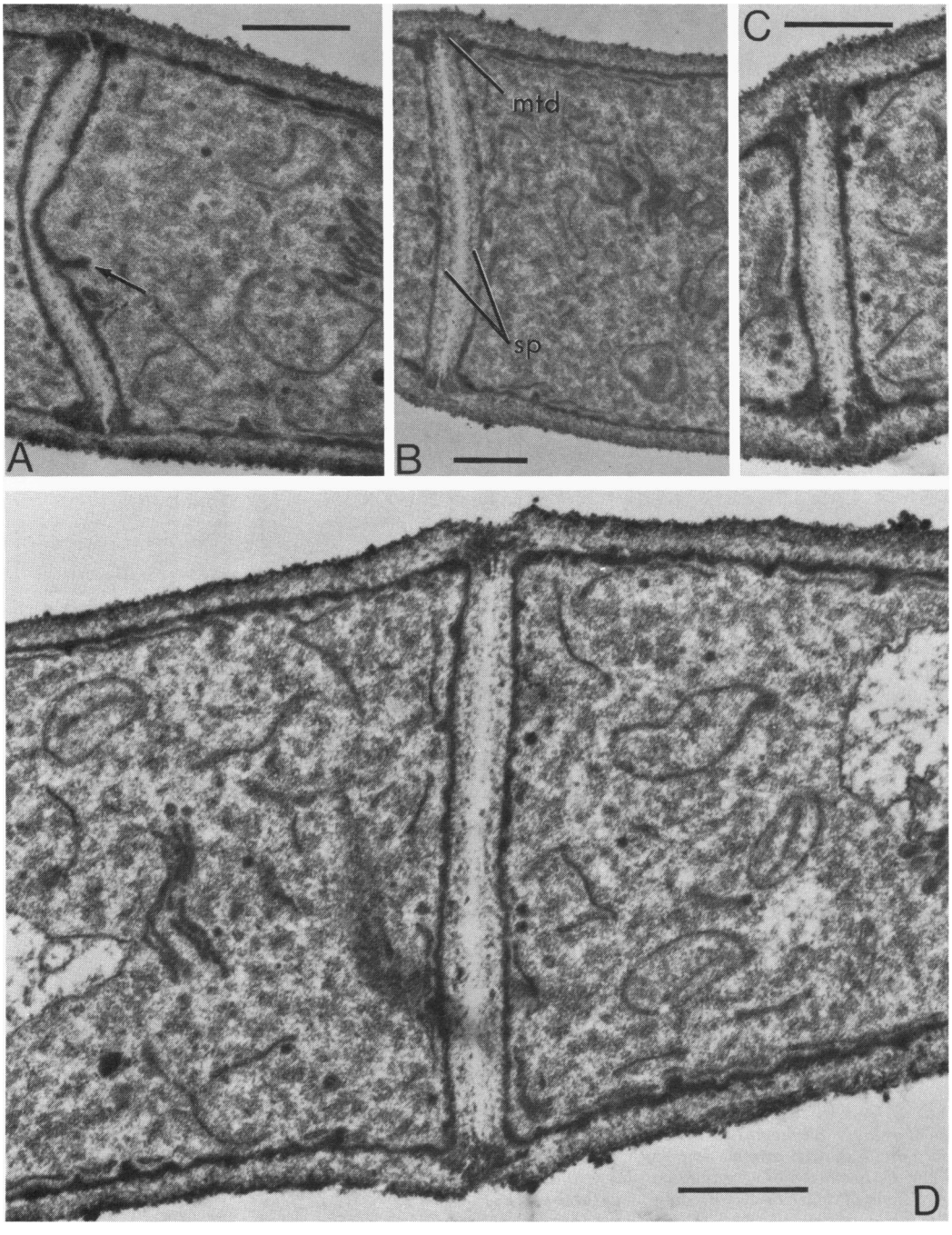

FIG. 4. *A, Scar plug (sp) layers now nearly grown together at middle of cell plate; fusion of dense body with dark layer continues (arrow); all magnification bars equal 0.5 μm. B, Material of fillet has migrated outwardly to become materiel triangulaire dense (mtd); sp layers now appear complete centrally and begin attachment to old cell wall behind mtd. C, Progressive outward movement of mtd. D, Erosion of outer surface of old cell wall and first appearance of mtd at outside (now fuscannel).*

sively thicker as the cell plate develops (Fig. 1C, D, E; 2B to 4B).

As the centripetally growing cell plate approaches closure, it becomes thickened by addition of material of intermediate transparency (scar plug [SP] layer [24], Fig. 3C, D), somewhat more transparent than the innermost parts of the old cell wall but still less transparent than the AR. The disposition of these new SP layers enhances the tapering appearance of the new cell plate (Fig. 3C, D). After closure of the pore and completion of the thickening process, the cell plate approximates a three-layer sandwich, with the inner electron-transparent layer (AR) and the two outer SP layers (Fig. 3E, F; 4A, B). Note, however, that the AR layer extends to make intimate contact with the original cell wall but that the two sandwiching SP layers are of lesser diameter, extending only to the fillet of electron-dense material. Finally, the AR layer appears to attain a greater diameter, partially replacing the old innermost cell wall layer and partially stretching the old cell wall at the site of the new, almost complete cell plate (Fig. 3E to 4D).

To this point, most of the visible changes occurred centrally, near or at the pore, with little change at the periphery where the electron-transparent layer of the new cell plate and the two electron-dense fillets lay apposed to the old cell wall. These annular fillets of electron-dense material then begin to migrate outwardly through the old cell wall and toward each other (Fig. 4A to 5C). They are replaced at their original position (Fig. 4D to 5C) by extensions of the SP layers, which also become apposed to the innermost portion of the old cell wall. At this point, the migrating electron-dense annuli approximate (in cross section) the dense triangular masses of Oulevey et al. (19; Fig. 1F). Concomitantly, fission begins by erosion of the outer cell wall region opposite the site of the newly completed cell plate (Fig. 4D to 5C). The simultaneous outward migration of the electron-dense material and the (presumably enzymatic) erosion of the original outer layers of the cell wall soon establish the electron-dense fuscannel at the outside surface of the dividing cells, just between the ridges of non-eroded original cell wall which remain as the fission scars (Fig. 1I; 5C). The migration of electron-dense material from the cytoplasm through the cell wall is then ended.

Fission is completed by centripetal erosion of the inner electron-transparent layer (AR) of the cell plate (Fig. 1I; 5C) and by restoration of the predivisional state of the cytoplasmic organelles.

DISCUSSION

The apparent changes of the vacuole and its vacuole-associated organelle at the initiation of cell division do not currently lend themselves to explanation. Although it may be reasonable to extrapolate from work on the vacuole of *Saccharomyces* (13, 26) and thus to suggest that the vacuole of *S. pombe* is a lysosome, we know nothing definitive about the vacuole-associated organelle. It has not previously been described and we hesitate to apply a more definitive name until something about its functions are known. Occasionally its border looks Golgi-like, and we are examining this further.

Our observations indicate no noticeable changes of the mitochondria or of the endoplasmic reticulum. In particular, we do not see the special proliferation and alignment of endoplasmic reticulum near the incipient cell plate seen by others (19) but are inclined to ascribe this difference in observation to differences between the two strains. We do see the reported (14) juxtaposition of mitchondria with dividing nuclei.

The occasional discovery of Golgi bodies in cells of *S. pombe* has been noteworthy (6, 19, 25); the infrequency of the observation has even led reviewers to disclaim their existence (17), except for the abnormal circumstances which accompany regeneration of the cell wall by protoplasts (6). However, Golgi bodies seem to appear in several pictures of synchronously dividing cells by Osumi and Sando (Fig. 3, 4, 9, 10, and 11 of reference 18), have recently been discussed in detail (10, 22), and we note many above. In the present circumstance, there is a parasynchronous cell division which initiates the logarithmic phase of growth between the 3rd and 4th h after inoculation (9). The high frequency of observation of Golgi bodies above would seem ascribable to their involvement with biosynthesis of cell plate materials, but the possibility of a correlation with overall high rates of glucan synthesis in these very long cells, whose rate of extension is known to be proportional to their lengths (8), should not be overlooked. (Most of the earlier observations of Golgi bodies in normal *S. pombe* cells [18, 25, and above] have occurred in strain NCYC 132.)

The relationship among the Golgi apparatus, the small electron-dense bodies, and the dark extra-cytoplasmic layer seems to be the typical "precursor-product" sequence (17). As noted above, the small dense bodies appear randomly distributed in the cytoplasm of this strain, and we see no evidence for locus specificity (19) near the cell plate.

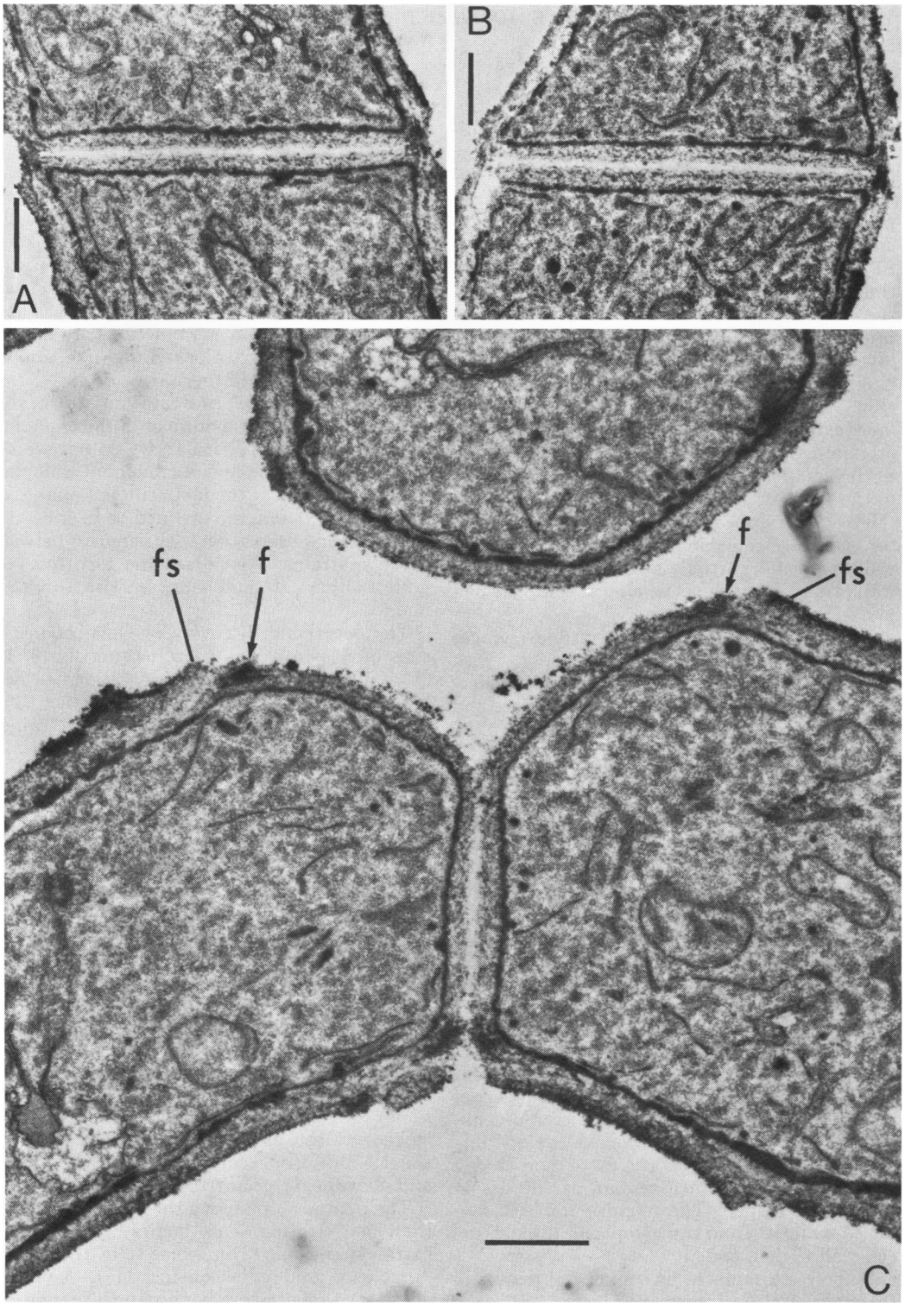

FIG. 5. *A, B, Progressive contact of scar plug layer with old cell wall; fuscannel (f) now external; all magnification bars equal 0.5 μm. C, Eccentrically dividing cell showing new fission scar (fs) and f at top, and slightly earlier stages at bottom; new ends of cell round as old annular rudiment material disappears.*

Fusion of the small bodies with the dark extra-cytoplasmic layer may be partly responsible for the appearance of variable thickness noted earlier (12) and above. However, glancing sections near the tip of the cell show ridge-like infoldings of the membrane and thus account for some of the variable thickness of this layer, similar to *Saccharomyces* (5). This layer in yeasts is probably either undifferentiated and multifunctional (various enzymes have been tentatively localized there, among them invertase [1] and an aminopeptidase fraction [21]) or is regionally differentiated with different functions at different sites around and along the cell perimeter. Its thickening into an annular fillet at the base of the rudimentary cell plate was noted early (12) and suggests a regional differentiation of function. One easily suggests that the dark material (materiel triangulaire dense of Oulevey et al. [19]), as it migrates outwardly to become the fuscannel, is implicated with the enzymatic erosion of the old cell wall and with biosynthesis at the edges of the SP layers when they extend to their final site of bonding to the old cell wall. Enzymically, the annular fillet of dark material cannot be simple.

The cell plate remains an enigma. It seems to be so very simple—an electron-transparent layer (AR) sandwiched between the two SP layers. Fission occurs at the AR layer, turgor pressure rounds the new cell ends, and finally the older tips reinitiate extensile growth (15). However, a few simple questions illustrate the depth of our ignorance. (i) What is the chemical composition of the AR layer? (ii) What is the enzymology of its biosynthesis? (iii) What is the enzymology of its splitting at fission? For the SP layers one may repeat the first two questions and ask additionally: (iv) how do the edges of the SP layers bind so firmly to the inner surface of the old cell wall? The available information is entirely cytological, including the differential electron opacity shown earlier (19) and above, the primuline fluorescence photographs of Streiblova et al. (23, 24), and the fact that 2-deoxyglucose is more heavily incorporated (B.F.J. and G.B.C., unpublished data) during cell plate biosynthesis than during extension.

The differential electron opacity of the various cell plate layers and of the old cell wall helps one to observe the growth process but says little about their compositions except to suggest that each layer may have a unique chemical or physical nature, or both. The differential incorporations of 2-deoxyglucose suggest only that the biochemistry of cell plate biosynthesis probably differs from the biochemistry of extension, useful information perhaps, but not surprising. Patterns of primuline fluorescence (24, and unpublished data of our own) may be interpreted now in terms of our electron microscope observations. Thus, the very bright fluorescence at the cell plate is indicative of staining of the AR, for it develops and disappears in centripetal patterns (23), much as the AR appears to grow centripetally, and then erodes centripetally. The rounding ends of the dividing cell (SP layer) have weaker fluorescence than any portion of the old wall except for those portions at older fission scars which were themselves derived from previous SP layers. Thus, the SP layer remains as an integrated, but almost certainly (chemically) different, portion of the cell wall. The consequences of this would surely complicate studies of the chemical structure of the glucans of *S. pombe* walls.

Bud scars of *Saccharomyces* cells seem to be areas forbidden to further budding (2). Although comparison is not quite direct, this does not appear to be true for *S. pombe*, for we find several cases of closely apposed fission scars with representatives of both possible geometries. Another comparison with *Saccharomyces* is in order. The observation that the region of the fission scar is a region of enhanced cellular diameter is quite comparable with the well-documented expansion of budding yeast adults which also occurs late in the budding cycle (reviewed in 7).

By either bright-field or phase-contrast microscopy, only the ridge of old cell wall and the enlarged cellular diameter can be seen at the site of an old fission scar; this ridge constitutes the classical division scar (15). However, an expanded definition would include the fuscannel and that portion of the wall formed from the SP layer of the cell plate. The latter can be identified only by its very dim primuline fluorescence by using the optical microscope, but has been well characterized with electron microscopy (24). The interesting thing about the fuscannel is that it appears in many electron micrographs of longitudinally sectioned *S. pombe* cells (12, Fig. 2; 18, Fig. 3; 19, Fig. 7; 22, Fig. 1; 24, Fig. 4; 25, Fig. 10) and once seen, seems a dominating exterior mark on the wall, although it has been mentioned only once (24). Its ontogeny as described above is most intriguing and would seem to merit careful cytochemical investigation.

Finally, the amount of movement of organelles, structures, and materials merits emphasis. The apparent movement by cyclosis of dense bodies from Golgi apparatus to dark layer is now held (17) to be a common pattern of

movement. The outward movement of the cell wall at the fission scar resulting in regionally enhanced cylindrical diameter is noncyclotic. Also noncyclotic is the movement of electron-dense material from the fillets at the base of the cell plate through the wall as the materiel triangulaire dense (19) to become the fuscannel. The mechanisms by which the cell organizes these morphogenetic movements remain to be described.

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