TERMINAL PHASE OF CYTOKINESIS IN D-98S CELLS

J. MICHAEL MULLINS and JOHN J. BIESELE

From the Department of Zoology, University of Texas, Austin, Texas 78712. Dr. Mullins' present address is the Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309.

ABSTRACT

The events leading to the completion of cytokinesis after the formation of the midbody and intercellular bridge in D-98S cells were studied with light and electron microscopy. Pairs of daughter cells corresponding to different stages of cytokinesis, as determined previously from time lapse films, were selected from embedded monolayers for serial sectioning. Separation of daughter cells is preceded by the reduction in diameter of the intercellular bridge from 1–1.5 μm to approx. 0.2 μm. Two processes contribute to this reduction: (*a*) The intercellular bridge becomes gradually thinner after telophase; a progressive breakdown of midbody structure accompanies this change; and (*b*) the more significant contribution to reduction in bridge diameter occurs through the localized constriction of a segment of the intercellular bridge. The microtubules within the constricted portion of the bridge are forced closer together, and some microtubules disappear as this narrowing progresses. The plasma membrane over the narrowed segments is thrown into a series of wavelike ripples. Separation of daughter cells is achieved through movements of the cells which stretch and break the diameter-reduced bridge. The midbody is discarded after separation and begins to deteriorate. Occasional pairs of daughter cells were found in which incomplete karyokinesis resulted in their nuclei being connected by a strand of nuclear material traversing the bridge and midbody. Such cells do not complete cytokinesis but merge together several hours after telophase. This merging of daughter cells coincides with the nearly complete breakdown of the midbody.

Cytokinesis in the metazoa may, on the basis of functional considerations, be divided into two phases. The initiation and development of the division furrow constitute the first of these phases. At the termination of furrowing, a narrow intercellular bridge with central midbody remains connecting the daughter cells (8, 13). The second phase of cytokinesis consists of the events involved with the severing of the intercellular bridge to actually separate the daughter cells (8, 24, 25). Relatively little attention has been given to the activities of this second phase through which cytokinesis is completed. Further, it is not known whether the prominent midbody plays any essential role in the completion of cytokinesis.

Electron microscope studies (6, 10, 19, 27, 29) have clarified the structural features of the midbody and its formation from the stem bodies during telophase, but no detailed electron microscope observations of the events after the formation of the midbody have been reported. Observations from time-lapse films of cultured HeLa (8) and D-98S (25) cells have shown that the final separation of daughter cells takes place through a definite

sequence of events involving recognizable changes in cell morphology. We report here an electron microscope study of the completion of cytokinesis in D-98S cells.

MATERIALS AND METHODS

D-98S cells, derived from human sternal bone marrow (2), were chosen for study on the basis of advantageous features of their morphology. The midbody is relatively large and can be readily identified and followed during time-lapse filming. Additionally, it was felt that the limited motility would allow subtle changes in the morphology of the intercellular bridge to be more easily recognized than in the situation where the bridge undergoes considerable stretching between actively moving fibroblasts. The basic similarity in form between D-98S cells and HeLa cells, the only other cultured cell for which the termination of cytokinesis has received detailed study (8), also provided the opportunity for comparison between the two.

Monolayer cultures of cells were maintained at 37°C in Eagle's Basal Medium supplemented with 10% calf serum. For time-lapse cinematography, cells were seeded into Sykes-Moore chambers modified according to the method of Freed (14). For electron microscopy cells were grown in 60-mm Falcon plastic petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). Cultures were fixed 24–36 h after being seeded. Fixation and embedding were carried out directly in the Falcon dishes, after standard methods for the flat embedding of cell monolayers (4, 9). All solutions were used at room temperature. Initial fixation was carried out for 30 min in a modified Karnovsky fixative (17) consisting of 3% glutaraldehyde, 4% formaldehyde, and 0.075% $CaCl_2$ in 0.1 M cacodylate buffer, pH 7.4. Cells were postfixed in 1% OsO_4, stained in 0.5% aqueous uranyl acetate, dehydrated through an ethanol series, and embedded in Epon (Luft's B formula (20) plus 2% DMP-30). After polymerization for 48 h at 60°C, the plastic wafers containing the cell monolayers were broken free of the plastic dishes and scanned with phase-contrast optics. Selected cells were photographed and circled, using a Zeiss object marker (Carl Zeiss, Inc., New York, N. Y.). Small areas of plastic containing the selected cells were sawed free of the wafers and glued to the tips of plastic rods for trimming and sectioning. Silver-to-gray serial sections were taken in the plane of the monolayer, using a Sorvall MT-2B ultramicrotome and a DuPont diamond knife (DuPont Instruments, Sorvall Operations, Newtown, Conn.). Sections were collected on carbon-Formvar-coated single-slot grids, stained with lead citrate and examined with a Siemens Elmiskop Ia electron microscope.

OBSERVATIONS

In the following descriptions the term "intercellular bridge" means the channel of membrane-bound cytoplasm that interconnects two daughter cells after the completion of furrowing. The word "midbody" will be used to denote only the phase-dense or electron-dense disc of material perpendicular to the axis of the intercellular bridge and located near its middle (Fig. 1). The term "half bridge" will be used to describe the portion of the intercellular bridge extending from the edge of either daughter cell to the midbody disc.

Early Midbody

Figure 1 illustrates the features of the midbody and intercellular bridge of D-98S cells as they appear directly after telophase. As seen in sections taken parallel to the long axis of the bridge, the midbody consists of an electron-dense band measuring from 800 to 900 nm in thickness. This band is composed of an amorphous, dark-staining material within which microtubules are embedded in a closely packed, parallel array. The dense, amorphous material of the midbody will be referred to as "matrix." The microtubules extend out from the matrix band on either side, passing the length of the bridge and extending into the daughter cells on either side. Studies of other cell types (3, 23, 27) have shown that many of the microtubules within each half bridge terminate within the midbody, interdigitating there with similar microtubules from the opposite half bridge. This feature cannot be readily discerned in our preparations due to the difficulty of tracing individual tubules in longitudinal sections. At the point where it is spanned by the midbody, the bridge has a diameter of from 1.5 to 2 μm, generally tapering to a slightly smaller diameter at its junctures with the daughter cells. At the area of association between the midbody and the plasma membrane of the intercellular bridge the membrane bulges outward, forming a small ridge. These ridges are not present at the earliest stages of midbody formation and appear to develop with time. Their formation is discussed below.

The midbody does not completely plug the intercellular bridge. While most of the bridge is occluded by the midbody, channels up to 250 nm in diameter are frequently found through the midbody (Fig. 5*b*). These channels are usually filled with small vesicles. Numerous small vesicles are also found interspersed among the microtubules within the bridge (Fig. 1), and elongate cisternae of rough or smooth endoplasmic reticulum are often aligned in parallel with the microtubules.

Reduction in Diameter of the Intercellular Bridge

Separation of D-98S daughter cells is preceded by a reduction in diameter of some portion of the intercellular bridge from an initial diameter of 1–1.5 μm to less than 0.5 μm. Two different processes contribute to this change in diameter. The first of these processes is seen when D-98S cells are followed with time-lapse cinematography for a period of 1–3 h after telophase. During this time the intercellular bridge becomes gradually thin-

ner. This decrease in diameter occurs across the entire length of the bridge, except at the midbody. The midbody retains its initial diameter and so projects progressively farther beyond the width of the bridge. The electron micrograph in Fig. 1 is representative of an early stage in this process; a later stage is seen in Fig. 2.

Examination of serially thin-sectioned midbodies shows that the portion of the midbody which extends beyond the bridge actually represents a ridge which completely encircles the bridge. The structure of this ridge is considerably different compared to the central part of the midbody. Within the ridge, microtubules are almost entirely absent (Figs. 1, 2, and 5). The few microtubules that are present are short lengths which typically do not extend out of the ridge into the bridge and which are oriented oblique to the tubules within the bridge (Fig. 2). Ridge formation seems to involve a time-dependent breakdown of the midbody microtubules, progressing from the periphery of the midbody toward its center. The absence of microtubules within the ridge could also be accounted for by a repositioning of microtubules as the ridge forms, but there are no indications that microtubule density increases in either half bridge in correspondence to the degree of ridge development. A layer of electron-dense material, varying from less than 5 nm to approx. 40 nm in thickness, underlies the plasma membrane of the ridge (Fig. 2). The rest of the interior of the ridge is loosely filled with similar dense material. This electron-dense substance probably represents matrix which remains after microtubules are eliminated from the ridge. Small, 20- to 80-nm diameter vesicles are frequently found clustered about the exterior of the midbody ridge (Fig. 5*b*), and in some cases such vesicles appear to be budding off of the ridge. The formation of vesicles from the ridge could account for the highly irregular contours of most of the midbody ridges observed.

In a few cases the gradual reduction in bridge diameter described above has been observed to progress to the point where both half bridges are less than 0.5 μm in diameter. Typically, however, a second, more rapid process completes the reduction in bridge diameter. This occurs through an apparently localized constriction of a segment of a half bridge (25). The time required for this activity, which we have termed "narrowing," varies from specimen to specimen. Four cell pairs for which the beginning and ending of narrowing could be distinguished with reasonable clarity from individual cine frames required approxs. 3, 4, 7.25 and 11 min to form a narrowed segment.

Seventeen half bridges fixed at various stages in the process of narrowing were studied by electron microscopy. The narrowed segments ranged from 0.3 to 2 μm in length and 0.2 to 0.6 μm in diameter. Of the 17 narrowed segments examined, three were as thin as 0.2 μm. None smaller was found, despite efforts to select cell pairs that might represent a later stage in the process, so it seems likely that 0.2 μm is the approximate minimum diameter to which narrowing can progress.

All of the narrowed segments examined shared a set of characteristic features which are illustrated by the intercellular bridge in Fig. 3. The plasma membrane over the narrowed section of a half bridge is formed into a series of wavelike ripples (Fig. 3*b*). The rippled contour is most clearly seen on medial sections. Sections grazing the membrane reveal the ripples as a series of alternating light and dark densities. Average crest-to-crest spacings of the ripples ranged from 34 to 43 nm for different specimens. No structures which might account for the rippled contour were evident beneath the plasma membrane.

The microtubules that pass through the narrowed segment on their way from the midbody to the daughter cells show a variation in spacing which suggests that they are being pushed closer together by the constricted plasma membrane of the segment. Microtubules from the periphery of the midbody curve inward, toward the center of the bridge, to enter the narrowed segment (Fig. 3*c*). Within the segment, tubules are packed more closely together than they are in the rest of the half bridge; they are aligned parallel to one another. As the microtubule bundle passes into the daughter cell it tends to splay apart, such that the parallel arrangement is lost (Fig. 3*c*). The ribosomes, numerous small vesicles and occasional cisternae of endoplasmic reticulum commonly found within the intercellular bridge are absent from the narrowed segments (Fig. 3*b*). These organelles are presumably displaced from the narrowed segment as the microtubules become clustered more closely together during narrowing.

As narrowing progresses, some of the microtubules within a narrowed segment disappear. Qualitatively, this is shown by the fact that narrowed segments ranging from about 450 to 200 nm in diameter appear to be equally tightly packed with tubules, suggesting that microtubules are eliminated from a narrowed segment to allow it to attain the smaller diameter. To quantitate this observation, counts were made of the number of microtubules present in medial sections through 13 narrowed segments ranging from about 200–615 nm in diameter. As shown in Fig. 4, the microtubule number varied directly with the diameter of the narrowed segment. If the microtubules within a narrowed segment were simply forced closer together as narrowing progressed, the number of tubules present in a section would be expected to remain constant or increase as the diameter of the narrowed segment became smaller. A decrease in microtubule number with decreasing narrowed segment diameter indicates that tubules are lost as narrowing takes place.

The portions of the tubules that are embedded in the midbody matrix appear to remain intact throughout narrowing. Some tubules emanating from all portions of the midbody can be traced into the narrowed segment, so the elimination of tubules does not seem to be restricted to either peripheral or central regions of the midbody. The decrease in tubule number could be brought about either through a removal of pieces of broken tubules

within the bridge or by microtubule depolymerization. The former should lead to an increase in tubule density on one or both sides of the narrowed segment. Since this was not observed, we infer that microtubule depolymerization is the more likely possibility.

The mechanism by which narrowing takes place is not clear from the results of electron microscopy. Careful examination of serial sections revealed no structures that could be interpreted as likely active agents for narrowing. Narrowing could be initiated either by the clustering together and progressive breakdown of the midbody microtubules, allowing cortical contraction to concomitantly decrease bridge diameter, or by active cortical contraction as the primary agent with the clustering and depolymerization of the microtubules occurring as a result of this activity. Our observations do not allow us to distinguish between these two possibilities. Narrowing does not depend on the maintenance of tension across the bridge by the daughter cells since an intact half bridge can still undergo narrowing after separation.

Separation of Daughter Cells

Once the diameter of some portion of the intercellular bridge has been reduced to approx. 0.2 μm, separation is brought about by various movements of the daughter cells which stretch the bridge at its thinnest part, causing it to break (25). As seen in the electron microscope the stretched section of the bridge is not a highly organized structure. The midbody microtubules remain associated with the midbody as stretching takes place, extending 1 to 3 μm into the stretched segment (Fig. 5*b*). Except for occasional short profiles, microtubules are not seen within the remainder of the stretched section, nor are they found associated with its juncture to the daughter cell. The only organelles consistently present in the stretched segment are small numbers of 5- to 6-nm-diameter filaments, aligned parallel to the bridge's long axis, and variously shaped small vesicles (Figs. 5*b*, and 6). The rippled membrane pattern characteristic of the narrowed segments is not seen at any point along a stretched half bridge. The diameter of the stretched bridge varies along its length, presumably reflecting the degree to which different parts of it have become attenuated. Diameters ranging from approx. 170 nm to as little as 25 nm have been measured.

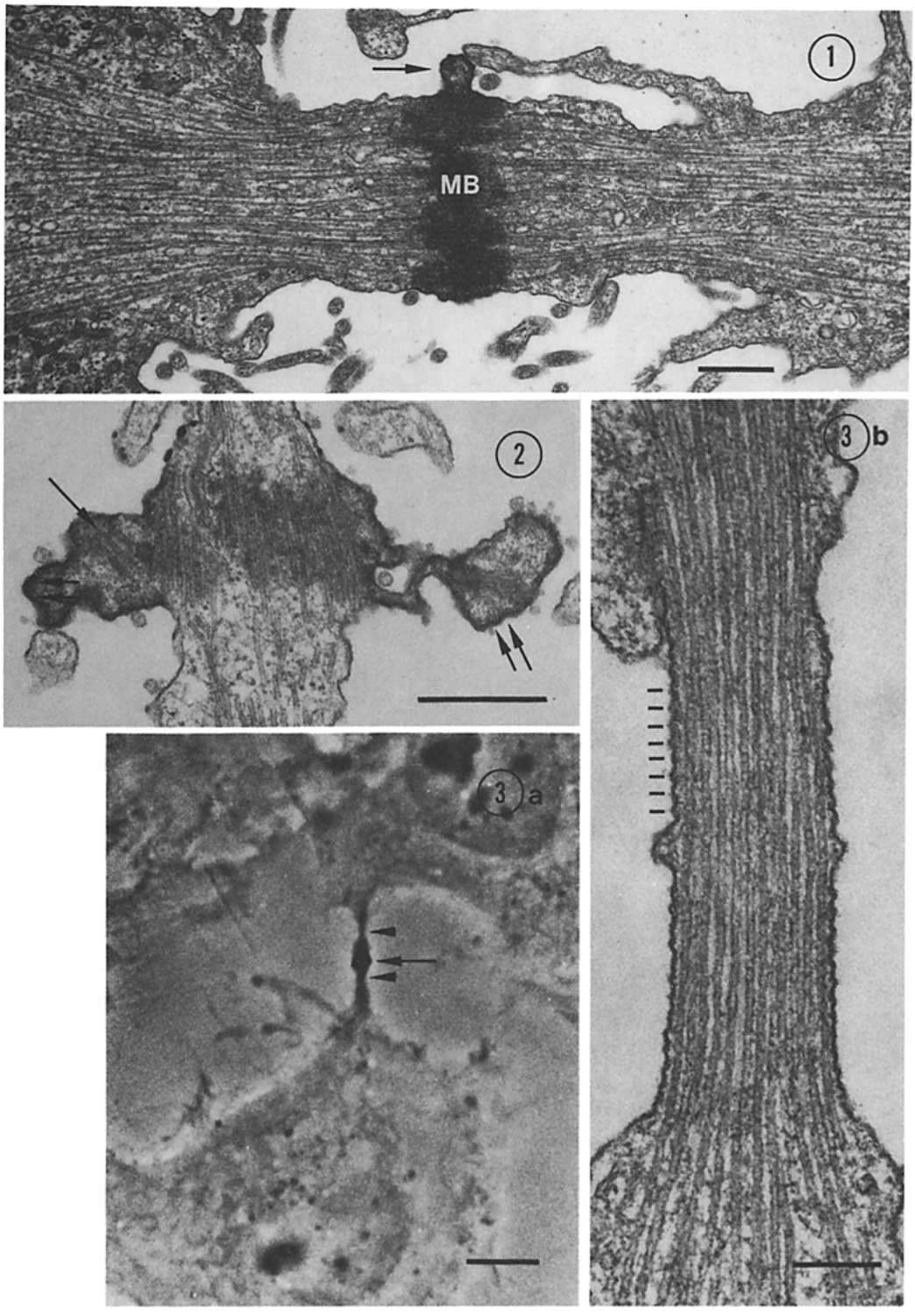
Postseparation Midbody

After cytokinesis is completed, the midbody remains attached to one daughter cell by the unbroken half bridge. In instances where the intact half bridge has not become reduced in diameter before separation, narrowing has been observed to take place immediately after separation. As a result, the postseparation midbody remains attached by a thin stalk, less than 0.5 μm in diameter. Once this condition is reached, cell movements, changes in cell shape, and the constant random motion of the midbody in and out of the plane of focus make it difficult to follow the eventual fate of the midbody by light microscopy. Observations from electron microscopy indicate that the midbody is discarded by the daughter cell to which it remains associated immediately after separation. Serial sections from 21 postseparation midbodies were examined. Of these, three were found to have an intact half bridge which could be traced to a definite connection with the daughter cell. In the other specimens no definite continuity between the half bridge and daughter cell was seen. In individual sections, areas were evident where the half bridge appeared to contact the edge of the cell. When such contacts were followed in serial section, however, they were found to represent areas of overlap or apposition between the half bridge and the cell periphery. In none of these specimens could an intact membrane be traced from cell to half bridge, nor was there any indication of a continuous cytoplasmic channel between the two. Additionally, the apparently discarded midbodies were found to be in various states of deterioration (Figs. 7 and 8). In some cases, intact microtubules were not present in either the half bridge or within the midbody matrix, although the channels through the matrix into which the microtubules had formerly inserted remained present (Fig. 7 and 8). The membrane around some specimens was clearly broken in places and was separated from its subtending layer of electron-dense material at the midbody ridge (Fig. 7).

Nuclear Continuity and Failure to Complete Cytokinesis

Occasional pairs of daughter cells are found in which incomplete karyokinesis has taken place. Daughter nuclei are physically continuous, being connected by a tubelike extension of the nucleus which runs through the intercellular bridge and midbody (Figs. 9*a*, 10*a*, 12*a*). This is the result of chromatin bridges which persist in the interzone during furrowing. The extension of the nucleus into a half bridge is seen in Fig. 9*b*; the nuclear extension is traced to the nucleus of the other daughter cell in the light micrograph of Fig. 9*a*. Where it passes through the midbody, the nuclear extension has a diameter of 90–200 nm but tends to broaden and flatten as it approaches the nucleus proper, being as wide as 700 nm. The DNA-positive staining noted by Malinin et al. (22) in midbodies of cultured mammalian cells is most likely another example of the phenomenon.

A pair of daughter cells previously observed to merge after the apparent breaking apart of the midbody (see Fig. 8 of reference 25) was found to be an example of nuclear continuity. To see whether there was any connection between this failure to complete cytokinesis and the occurrence of nuclear continuity, additional pairs of daughter cells which displayed definite signs of nuclear continuity were selected for observation. Of 11 cell pairs, one pair completed cytokinesis after approx. 7.5 h of observation. The 10 other cell pairs failed to complete cytokinesis. After periods of time ranging from about 1 to 9 h, the cells merged (Fig. 10). The beginning of merging was marked either by a change in shape of the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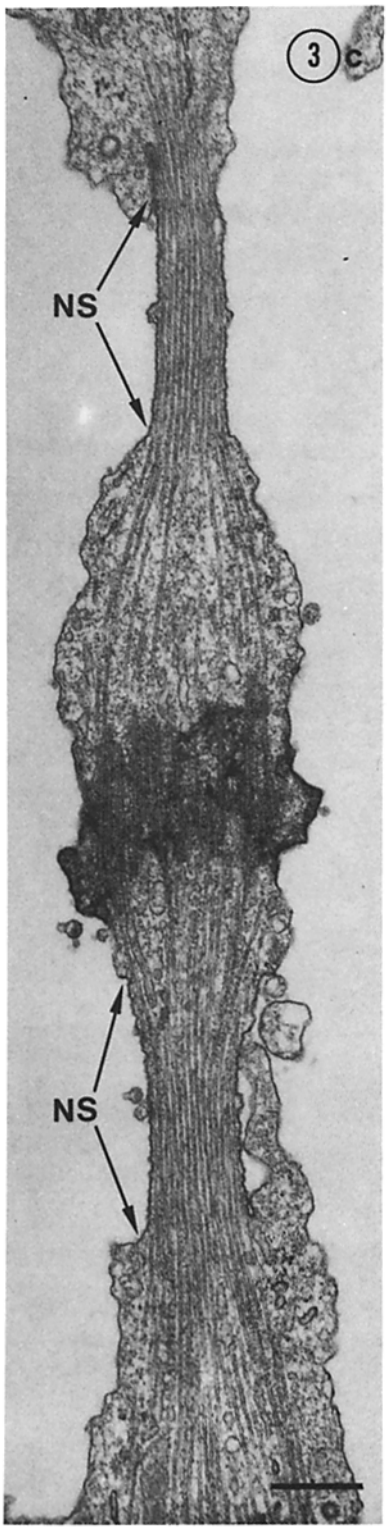


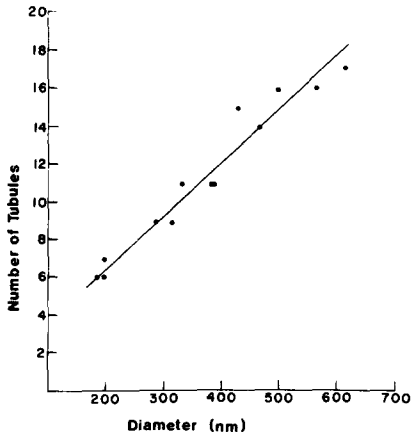


FIGURE 4 The number of microtubules present in medial sections through each of 13 narrowed segments (ordinate) is plotted against the diameter of the segment (abscissa).

midbody, sometimes appearing as though it had actually broken apart at one point, or by the apparent loss of contact between the midbody and one side of the bridge. At this stage the cells stopped moving in opposition to each other, the bridge began to broaden, and the cells began to move together (Figs. 10*b* and *c*). After 0.5–1.5 h the situation shown in Fig. 10*d* resulted; at the center of the single cytoplasmic mass the two nuclei lay adjacent to each other. Careful focusing showed the nuclei to be connected by a short neck about 1.5–2.5 μm wide.

FIGURE 1 Intercellular bridge and midbody (*MB*) fixed shortly after telophase. Microtubules are absent from the small midbody ridge (arrow), seen at only one edge of the midbody in this section. Bar, 0.5 μm. × 22,200.

FIGURE 2 Irregular contour of a large midbody ridge is shown in this micrograph. Single arrow indicates skew microtubules within the ridge. Double arrows indicate places at which the thin layer of electron-dense material beneath the plasma membrane of the ridge can be clearly seen. Bar, 0.5 μm. × 39,000.

FIGURE 3 Intercellular bridge with each of its half bridges at a different stage of narrowing. (*a*) Light micrograph of the cells embedded in plastic. An arrow labels the phase-dense midbody; arrowheads mark the narrowed segments. Bar, 5 μm. × 2,400. (*b*) A section through the upper narrowed segment. The small, dark lines mark the rippled pattern of the plasma membrane at one location. Bar, 0.2 μm. × 65,500. (*c*) A section through both narrowed segments (*NS*), the upper with a diameter of approx. 350 nm and the lower of 450 nm. Bar, 0.5 μm. × 26,700.

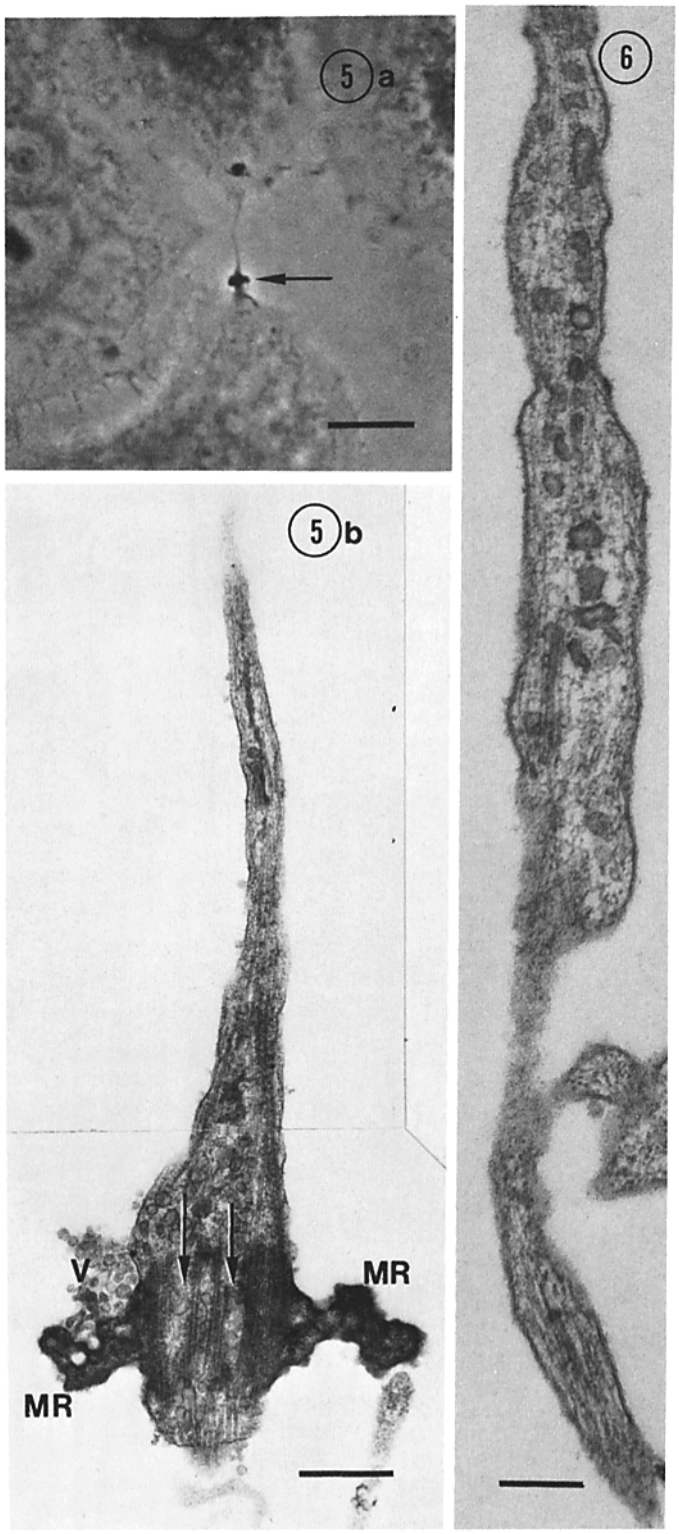


FIGURE 5 Example of a half bridge stretched between daughter cells. (*a*) Light micrograph of the cells embedded in plastic. The stretched half bridge is seen as a thin strand extending from the midbody (arrow) to the periphery of the upper daughter cell. Bar, 5 μm. × 2,200. (*b*) Microtubules extend from the midbody into the stretched half bridge for a distance of about 2.5 μm. Small vesicles (*V*) are clustered near part of the large midbody ridge (*MR*). Arrows indicate cytoplasmic channels through the midbody. Bar, 0.5 μm. × 24,500.

FIGURE 6 A high magnification view of part of a stretched half bridge. Bar, 0.2 μm. × 54,500.

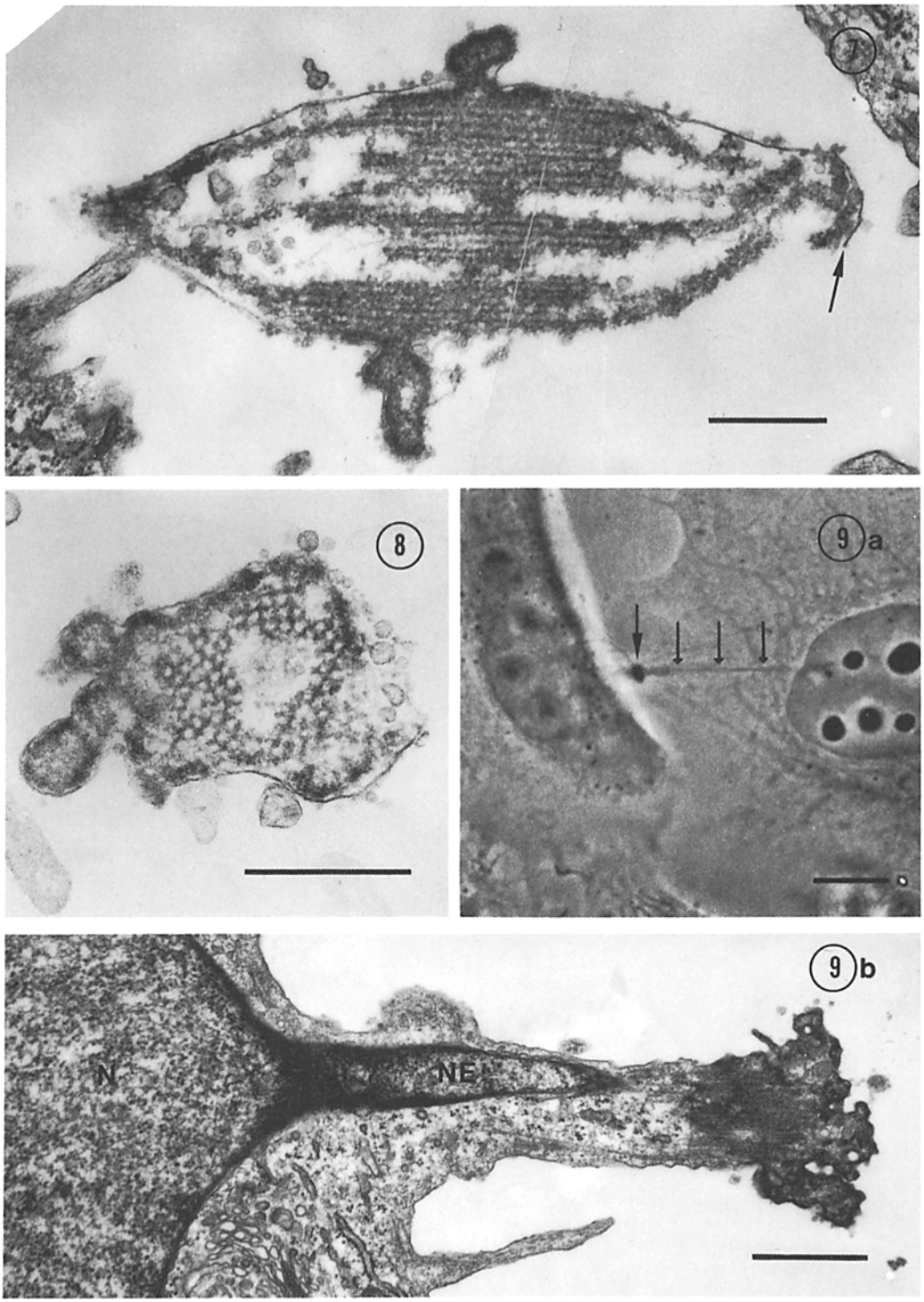


FIGURE 7 A discarded midbody which has begun to deteriorate. Note that intact microtobules are not discernible within either the midbody matrix or either half bridge. The arrow indicates a free end of the broken plasma membrane. Bar, 0.5 μm. × 35,000.

FIGURE 8 Cross section through a deteriorating, discarded midbody in which the matrix remains intact after the loss of the midbody microtubules. Bar, 0.5 μm. × 49,500.

FIGURE 9 Example of nuclear continuity. (*a*) Light micrograph of the embedded cells. Small arrows mark the thin strand of nuclear material extending from the nucleus of the right daughter cell into the midbody (large arrow). Bar, 5 μm. × 2,200. (*b*) The nuclear extension (*NE*) is shown extending from the nucleus (*N*) of the left daughter cell into the left half bridge. Bar, 1 μm. × 19,200. In serial sections through this specimen the nuclear extension was traced from one nucleus to the other.

Despite this connection the nuclei did not unite into a single structure. 10–18 h after the beginning of merging, two distinct nuclear profiles were still observed. It is estimated that 7–11 h or more may elapse between telophase and merging.

Before merging, each cell pair had undergone the reduction in diameter of both half bridges and displayed a prominent midbody ridge. The sorts of cell movements that normally lead to the breaking of the bridge were observed to take place, with no effect, up to the commencement of merging. Electron micrographs of the thin half bridges show them to be almost entirely filled with the nuclear extension and to be devoid of microtubules (Fig. 11). These observations suggest that the nuclear material within the bridge strengthens it sufficiently to prevent its breakage by movements of the daughter cells. No other structures are present which would seem likely to cause the delay in separation Further, of 43 cell pairs observed during the completion of cytokinesis, only those with nuclear continuity were observed to undergo such a failure of cytokinesis. To control for the possibility that prolonged exposure to light might be causing these daughter cells to merge, 7 of the 11 cell pairs displaying nuclear continuity were exposed only briefly to light for photography, at intervals of 15–45 min, until after merging had commenced.

The time-dependent deterioration of midbody structure involved with ridge formation continues to take place during the prolonged period of time in which the intercellular bridge remains intact. Fig. 11 shows a midbody that has undergone extensive breakdown. Microtubules are present in the lower portion of the midbody but are absent in the rest of it. The entire midbody is essentially reduced to the appearance of a midbody ridge. The extent to which this process proceeds is illustrated by the midbody of Fig. 12. The appearance of this specimen is unusual in that the small (approx. 1 μm diameter) midbody lacks any indications of ridge development, and the intercellular bridge is not clearly delimited from the periphery of either daughter cell (Fig. 12*a*). These features are characteristic of the changes in shape which accompany the beginning of the merging of daughter cells, so this cell pair was sectioned to examine the condition of the midbody. The midbody was found to be essentially degraded (Fig. 12*b*). Only a layer of electron-dense material on the inner surface of the plasma membrane marks the location of the midbody. The interior of the bridge beneath the midbody is open, as is indicated by the bundle of 5- to 6-nm-diameter filaments spanning it. The significance of these filaments is not known. Another pair of cells which was clearly in the process of merging showed no evidence of any midbody remnant by either light or electron microscopy.

DISCUSSION

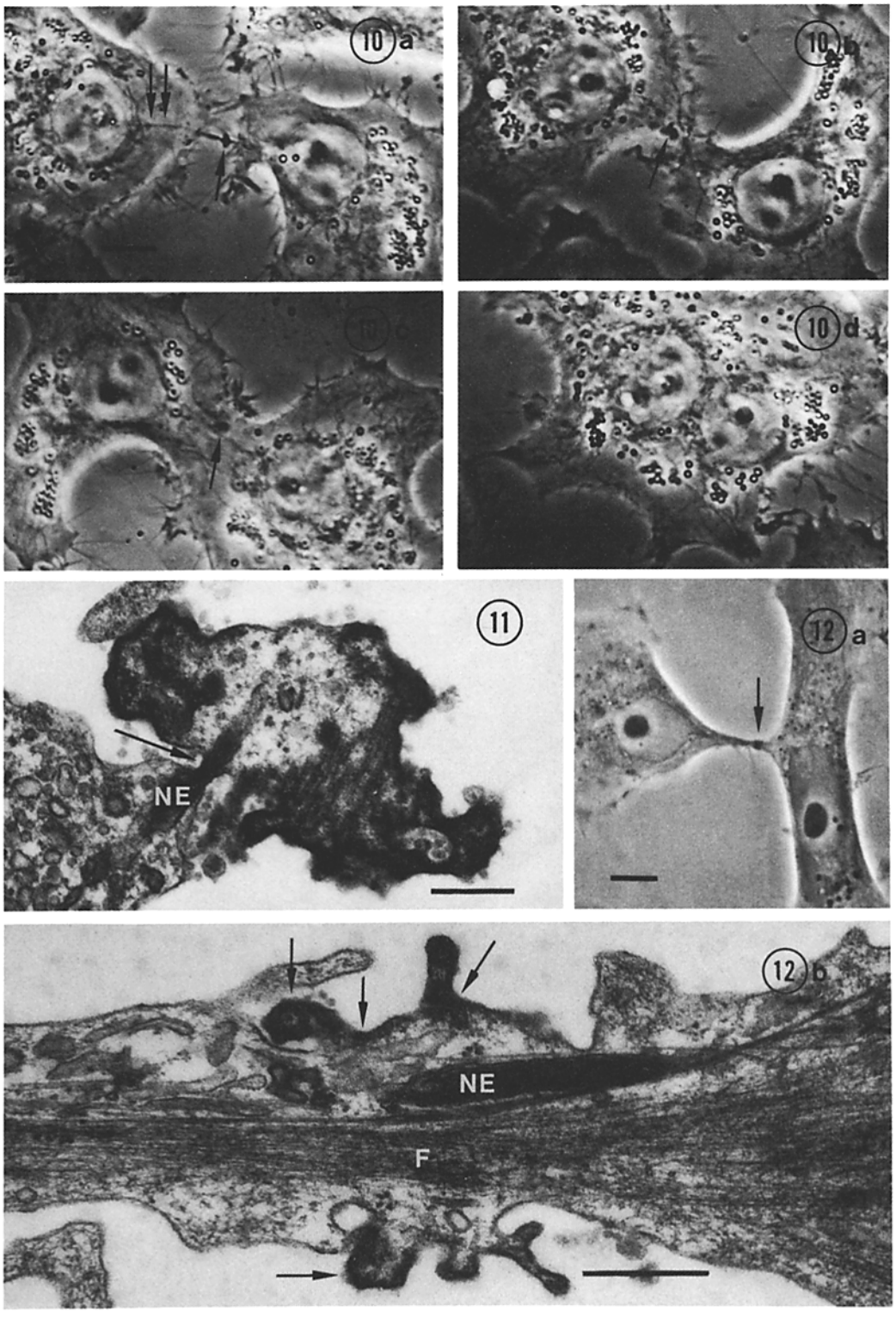
Our observations show that the completion of cytokinesis in D-98S cells involves a definite pattern of changes in the structure of the midbody and intercellular bridge corresponding to the sequence of events observed by light microscopy and described previously (25). Some of the events described constitute new observations; some have been noted in the work of others, but in less detail (discussed below). The important features of this study are the facts that these events can now be understood in terms of a time sequence progressing from the termination of furrowing through the separation of daughter cells, and that some understanding of the significance of these changes to the overall process of cytokinesis is now possible.

The reduction in bridge diameter which pre-

FIGURE 10 Sequence illustrating the failure of cytokinesis characteristic of cell pairs with nuclear continuity. The midbody is indicated by a single arrow in the first three frames. (*a*) Bridge and midbody are intact. The nuclear extension (double arrows) is seen as a phase-dense line running from the nucleus to the base of the intercellular bridge in the left daughter cell. (*b*) Merging of daughter cells has just begun. + 6h 24 min. (*c*) Later stage of merging, +6h 39 min. (*d*) Daughter cells have completely merged. +7h 32 min. Bar, 5 μm. × 1,600.

FIGURE 11 Advanced state of midbody degradation where separation has been delayed by nuclear continuity. This section passes through the thin left half bridge (arrow; diameter approx. 70 nm) which is almost completely filled with the nuclear extension (*NE*). The intact right half bridge is out of the plane of section. Bar, 0.3 μm. × 42,200.

FIGURE 12 Daughter cells judged to be at the initial stage of merging. (*a*) Light micrograph of the embedded cells showing the small, square-shaped midbody (arrow). The nuclear extension is seen as a peak off of the nucleus of the left daughter cell, pointing toward the intercellular bridge. Bar, 5 μm. × 1,400. (*b*) Midbody consists of only a plaque of electron-dense material (arrows) underlying the plasma membrane of the bridge. Only part of the nuclear extension (*NE*) is seen in this region. A bundle of filaments (*F*) extends through the region of the midbody. Bar, 0.4 μm. × 49,900.


10 a
10 b
10 c
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12 a
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F

cedes separation is presumably necessary to produce a bridge sufficiently diminished in size and strength so that movements of the daughter cells are adequate to stretch and break it. This idea is supported by the observation that before a significant reduction in bridge diameter (to 0.5 μm or less) cell movements identical to those which eventually result in separation merely serve to increase the overall length of the intercellular bridge. The midbody microtubules should make the bridge rigid to some degree, providing cytoskeletal support as do groupings of parallel microtubules in other systems (28, 31). The extent of such support may be limited due to the absence of the organized arrays of intertubule bridges noted in those systems. Nonetheless, the rigidity of the tubules should resist any decrease in bridge diameter that would cause them to bend inward toward the axis of the bridge. Moreover, the minimum diameter to which the bridge is reduced will depend upon the number of microtubules present and the area they occupy when forced to a minimum spacing. Narrowing and the events of midbody ridge development appear to be mechanisms through which the resistance provided by the midbody microtubules to decreases in bridge diameter is overcome. In the case of ridge development, this is achieved through a progressive breakdown of the microtubules across the entire length of the bridge, beginning at the periphery of the midbody and progressing toward its center. Narrowing, on the other hand, involves a more rapid, localized activity through which the microtubules in a half bridge become spaced closer together and are broken down.

The reduction in bridge diameter associated with midbody ridge development is probably of general significance for the termination of cytokinesis. While detailed descriptions of the process have not been reported previously, the early formation of the midbody ridge has been noted for HeLa cells (8, 27), and ridges of various degrees of development are evident in published micrographs of human and rat erythroblasts (6, 16). The swellings noted at the midpoints of the open bridges linking cells in clusters of oocytes or spermatocytes in many organisms (5, 7, 11, 12, 18, 21, 26, 32) are probably also examples of midbody ridge formation. Although midbodies are lost from such bridges before cytokinesis is completed, a layer of electron-dense material remains beneath the plasma membrane at the center of the bridge. This condition resembles that seen in D-98S cells where similar electron-dense material underlies the membrane at the midbody ridge.

Narrowing has not been reported for any other cell type, so the question as to whether it is a unique feature of D-98S cells or is of widespread occurrence remains to be answered. Byers and Abramson did not observe narrowing in their time lapse study of HeLa cells (8), but it is not clear from their paper how the intercellular bridge became reduced in diameter before separation. Narrowing could conceivably have taken place but not have been noticed due to the pronounced blebbing of the cells. From limited light microscope observations of HeLa and PtK1 cells, we have noted intercellular bridges which appear to have undergone narrowing, suggesting that narrowing will be found to be a characteristic feature of the completion of cytokinesis.

Final separation of daughter cells seems to involve only the stretching out of the intercellular bridge until its breaking point is reached. The observations presented here show that for D-98S cells the midbody is discarded and deteriorates once cytokinesis is completed. Paweletz (27) reported the loss and disintegration of the midbody after the completion of cytokinesis in HeLa cells. Other investigators have suggested the loss of the midbody after cytokinesis (1, 16) but did not base their conclusions on serial sections, which are necessary to determine whether or not this has actually occurred. It is interesting to note that the durable matrix may be the only component of the mitotic apparatus that is not conserved by the cell. The mechanism through which the midbody is discarded is not clear, but presumably involves contractile activity at the juncture of the intact half bridge and its daughter cell to pinch off the midbody from the cell periphery. It is possible that in some instances, particularly where cell movement is restricted, the same sort of mechanism may function in the initial breaking of the intercellular bridge to separate daughter cells rather than a stretching of the bridge.

Cells displaying nuclear continuity fail to complete cytokinesis. The facts that ridge development continues to take place in specimens in which separation is delayed by nuclear continuity, and that cells which appear to be in the process of merging lack any organized midbody structure suggest that it is the eventual breakdown of the midbody that leads to the widening of the bridge. Observations by Fawcett et al. (12) are consistent with the idea that the midbody is necessary to

stabilize the intercellular bridge. They noted that in spermatocytes dissected out of cat and guinea pig testes the intercellular bridges between daughter cells gradually widened until the cells merged into a single cytoplasmic mass. This instability is of interest because the bridges connecting germ cells lose their midbodies after telophase (18, 21), suggesting that once these cells were dissected free of surrounding interstitial tissue there was nothing to prevent regression of the furrow.

Considering these observations, we suggest a hypothesis for the functional role of the midbody in the completion of cytokinesis. The contractile ring, apparently the agent responsible for furrowing, disappears at the end of telophase (15, 30). We envision the midbody as serving to stabilize the furrow once the contractile ring is lost. The apparent ability of the matrix to bind to both the plasma membrane of the furrow base and the midbody microtubules would allow it to act as a "glue," cementing these structures into a stable configuration.

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