Membranes in the Mitotic Apparatus of Barley Cells

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ABSTRACT Membranes in the mitotic apparatus have been investigated ultrastructurally in dividing cells of barley (*Hordeum vulgare*). After osmium tetroxide-potassium ferricyanide or ferrocyanide postfixation (OsFeCN) of material that had been fixed in glutaraldehyde in the presence of Ca^{++}, the nuclear envelope (NE)-endoplasmic reticulum (ER) complex is selectively stained, permitting observations on the cellular pattern and structural ramifications of this membrane system that have not been previously recognized. Specifically, it is observed that during mitosis the NE-ER forms a continuous membrane system that ensheathes and isolates the mitotic apparatus (MA). Elements of ER progressively accumulate in the region of the spindle pole, becoming most concentrated by early anaphase. Within the MA itself, there are striking spindle-membrane associations; in particular, tubular elements of predominantly smooth NE-ER invade the spindle interior selectively along kinetochore microtubules. The membrane elements at the pole and surrounding the MA consist of tubular reticulum and fenestrated lamellae. Membranes of the MA thus resemble in considerable detail the tubular network and fenestrated elements of the sarcoplasmic reticulum of muscle. It is suggested that the NE-ER of the dividing barley cell may function in one or both of the following ways: (*a*) to control the concentration of free Ca^{++} in the MA and (*b*) to serve as an anchor for chromosome motion.

Membranes are common constituents of the mitotic apparatus (MA) of dividing cells. The classic work of Porter and Machado (22) revealed an association of endoplasmic reticulum (ER) with the MA in dividing cells of onion root tip. Since that time, other studies have appeared that have shown membranes, often of smooth ER, within the MA in both plants (9) and animals (13). In higher plants it is especially noteworthy that aggregations of ER occur at the spindle pole (12, 13), and tubular or lamellar extensions of this system have been observed in the spindle interior (13). Despite the numerous observations of membranes in the MA, these elements have received relatively little attention in the form of a concerted effort to understand what, if any, their role in mitosis might be. Instead, the focus has been on the presumptive motile elements, the spindle microtubules and, more recently, the actin microfilaments, since they appear to establish the structure of the MA and to cause the movement of chromosomes.

With our increasing awareness of Ca^{++} as regulators of motility in muscle (4, 11) and nonmuscle systems (3), the MA-associated membranes acquire new significance. It seems quite plausible that these membranes, like the sarcoplasmic reticulum (SR) of muscle, contain reservoirs of calcium that can be released and resequestered, thus altering the ion concentration in nearby regions of the MA (9, 10, 13). Processes such as microtubule assembly and actomyosin-mediated sliding that are known to respond to changes in calcium concentration could be regulated. In addition, membranes might be important structural elements in the MA. It has been suggested that they may serve as anchors for spindle fibers (8, 10).

Because of the potential importance of membranes, especially the ER, to the form and function of the MA, we have been reinvestigating these elements. A new technique, previously applied to studies of the SR (5), has allowed us to specifically stain the NE-ER of dividing cells and to obtain a more complete picture of the pattern of ER and its changes in distribution during mitosis. The technique has also permitted us to observe structural ramifications and associations heretofore not recognized. In particular, it is observed that in some plant species a continuous tubular network of ER extends from the pole into the spindle interior specificially along kinetochore microtubules.

This paper constitutes the first of two on membrane structure and its role in mitosis. The present one considers the detailed structure of ER in the MA, while the companion one (29) reveals, through the use of potassium antimonate, the presence and location of bound calcium in dividing cells.

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MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* cv early bonus) were germinated on moist filter paper for 48–72 h at 20°C. Both leaf and root tissues were prepared for ultrastructural examination. To easily find actively dividing leaf cells, germinating seedlings in which the coleoptiles were 12–18 mm long were selected. The entire shoot tissue was then excised and the basal 2 mm discarded. The next 2 mm was then excised, the coleoptile sheath removed, and the leaf segments were sliced longitudinally and placed as quickly as possible into fixative. It had been learned from personal communication with Dr. Eduardo Zeiger, Stanford University, that the leaf material isolated in the above manner would contain a large number of dividing cells of the stomatal complex, including those in the process of forming guard cells, guard mother cells, and subsidiary cells. Dividing leaf mesophyll cells also occur in this region with a considerable frequency. Dividing cells of the root were obtained by excising the apical meristems and placing them immediately into fixative.

Normal Fixation

Excised tissue was fixed in 2% glutaraldehyde buffered with either 0.05 M phosphate (pH 7.0) or 0.05 M cacodylate (pH 7.4) for 1–2 h. After fixation, tissues were washed in buffer and postfixed in 1% buffered osmium tetroxide for 1 h.

OsFeCN Postfixation

Tissues to be treated with the osmium tetroxide-potassium ferricyanide or ferrocyanide reagent (OsFeCN) (5) were fixed in 2% glutaraldehyde in 0.05 M cacodylate buffer containing 5 mM calcium chloride. The pieces were then washed in buffer plus calcium chloride and postfixed in a buffered mixture of 1% osmium tetroxide and 0.8% potassium ferricyanide [$K_3Fe(CN)_6$] or ferrocyanide [$K_4Fe(CN)_6 \cdot 3H_2O$] for 2 h. After this treatment the tissue was then further stained with 2% aqueous uranyl acetate for 2 h. Since it had been reported by Forbes et al. (5) that removal of calcium chloride from the fixation medium abolished the staining of SR in cardiac muscle, the same experiment was tried on the root tissue. In these studies the tissues were treated identically with the procedures listed above, except that calcium chloride was not included or phosphate buffer was substituted for cacodylate.

After these various fixative schedules, the tissues were dehydrated in a sequence of 2-methoxyethanol, ethanol, and propylene oxide, and embedded in an Epon-Araldite plastic mix. Sections cut with a diamond knife were mounted on carbon-Formvar-coated 1- to 2-mm open hole grids and examined either unstained or stained with lead citrate. Examination was carried out with a Zeiss EM-10A, a Siemens 102, a Philips 300, or a JEOL 100 CX electron microscope.

RESULTS

Staining of the NE-ER network by OsFeCN

The OsFeCN procedure, which has previously been used to contrast the SR, and to a limited extent the transverse axial tubular (T) system of cardiac muscle (5), specifically stains the NE and ER of barley. The other membranes and organelles are visible by this procedure but only the NE-ER complex acquires the marked electron density.

A paper in preparation will consider the characteristics of this reaction in greater detail, but a few of the salient features are briefly mentioned below. OsFeCN contrasts the leaflets of the membranes and, in addition, fills the cisternal space with an electron-dense product. It is largely this space-filling property of the reaction that makes it easy to recognize elements of ER even when they are sectioned tangential to their surface. Segments of membranes may be distinguished as ER and identified as part of a much larger reticulate-tubular network despite their appearance as vesicles within the thin section. It is important to note that the staining appears to be dependent upon the presence of calcium in the fixative because its exclusion or the use of phosphate buffer, which precipitates calcium, produced no staining.

Some problems and inconsistencies do exist, however, with this reaction. For example, the NE-ER will not usually be stained in all cells of a given block of tissue. In barley the NE-ER in ~50% of the root cells becomes stained, whereas in only ~25% of the leaf cells are these membranes selectively contrasted. No particular cell type is excluded; rather, it looks as if the partial staining results from a failure of the OsFeCN reagent to penetrate all cells, especially those deep in the tissue. Partial staining is also occasionally observed within a single cell. Although generally, if any part of a cell's NE-ER becomes stained, it all stains, examples have been noted in which the ER on one side of the cell will be more intensely stained than that on the opposite side. Again, variable penetration seems to be the problem because the heavily stained side is usually next to a cell that is completely stained.

Pattern of NE-ER during Mitosis

The staining of the NE-ER complex with OsFeCN permits us to follow with considerable clarity the changes in these membranes throughout mitosis. During prophase the NE-ER complex shows little or no organization that suggests the oncoming events of mitosis. However, with the breakdown of the NE at prometaphase, elements of ER, along with fragments of NE, aggregate in the region of the pole (Fig. 1). Also at this early phase of mitosis, some stained membranes protrude into the spindle interior.

The distribution of membranes at metaphase is shown in Fig. 2. Elements of NE-ER appear to progressively accumulate at the poles, and to extend along the sides of the spindle virtually surrounding the MA. In addition, stained membranes protrude from the pole into the spindle interior. These intrusions are especially noteworthy as they occur specifically along kinetochore microtubules (see next section of Results for more detailed observations).

One of the most conspicuous features of the spindle-associated membranes is the presence of aggregations of NE-ER in the region of the spindle pole (Figs. 2 and 4). In Fig. 2, swirls of ER lamellae transverse between the cytoplasmic cortex and the MA, forming prominent polar caps. At higher magnification (Figs. 3 and 4), OsFeCN staining reveals some important fine-structural detail within these membranes. Most apparent are the numerous pores or fenestrations. Observed in lamellae sectioned normal to their surface (Fig. 3), the fenestrations, like nuclear pores, pass through both layers of the ER but, unlike nuclear pores, are of variable diameter (40–100 nm). An electron-lucent region can be observed demarking each pore site, suggesting that the ground cytoplasm, consisting mainly of ribosomes, is excluded. In face views (Fig. 4) of sections passing tangentially through the membrane surface, it is evident that the fenestrations are somewhat irregular in outline. It is also apparent that membrane morphology is quite variable because regions continuous with the fenestrated lamellae consist instead of a highly reticulate tubular network.

Anaphase is characterized by an even more concentrated accumulation of ER in the spindle pole than was observed at metaphase. A striking example is shown in Fig. 5 in which the poleward side of the chromosomes is virtually filled by ER membranes. Although an occasional organelle may become entrapped in this membrane system, usually organelles are excluded just as they are eliminated from the spindle structure itself. By telophase, the polar aggregation of ER begins to disperse and the NE reforms (data not shown). Because cell plate formation is being dealt with in a separate paper, ER distribution during cytokinesis will not be considered in this study.

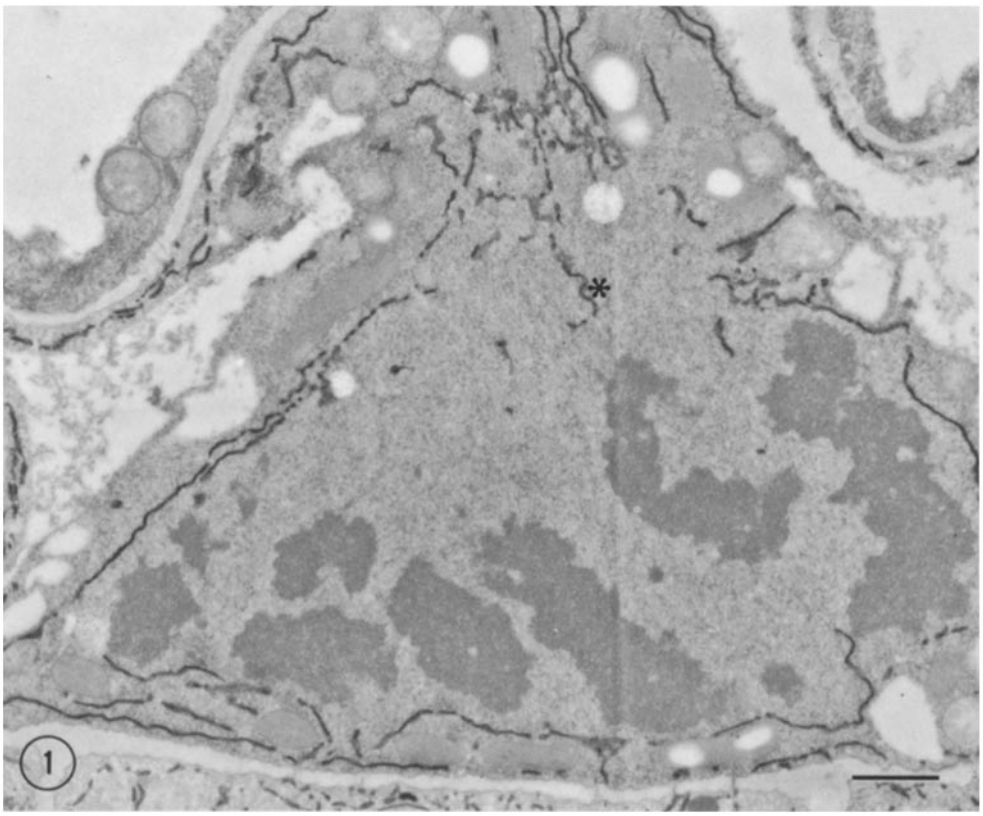


FIGURE 1 Early prometaphase in a subsidiary cell. NE-ER membranes, stained by OsFeCN postfixation, are beginning to aggregate in the spindle pole shown in the upper part of the micrograph. Some stained membrane has invaded the spindle interior (*). Bar, 1 μm. × 12,000.

Membrane-Microtubule Relationship

One of the most exciting observations to emerge from this study is the specific association of NE-ER with the kinetochore fibers. In Fig. 2, tubular profiles of ER extend into the spindle only along the kinetochore fibers. An enlargement of the upper right kinetochore in Fig. 2 is shown in Fig. 6 along with a serial section mate (Fig. 7). These micrographs show the kinetochore tubules faintly and indicate the close apposition between these tubules and the tubular NE-ER membranes. By comparing these two figures (Figs. 6 and 7), one can more readily appreciate the reticulate nature of the membranes in the third dimension and find evidence that the NE-ER forms a single connected system from the pole nearly to the point of microtubule attachment at the chromosome. When sections are stained with lead citrate, the non-NE-ER structure within the cell becomes more apparent, making it easier to observe the membrane-microtubule juxtapositions within the kinetochore (Fig. 8). Membranes here, and in Figs. 6 and 7, align themselves with the microtubules and show examples of close structural association.

That these images are not an artifact of the OsFeCN procedure is shown in conventionally prepared material (Fig. 9). Membranes, although not so distinct, nevertheless are seen to be closely associated with kinetochore tubules and to extend into the MA to the point of microtubule-chromosome attachment.

In addition to the NE-ER complex it is apparent that other kinds of membranes are present in the MA. Careful comparison of conventionally fixed material and that treated with OsFeCN allows us to make a distinction between the tubular-reticular NE-ER membranes and nearby spherical vesicles. In conventionally fixed material, the vesicles are more sharply delimited than the ER membranes, and their contents stain more densely (Fig. 9). In OsFeCN-treated material, the contents of these vesicles are almost electron transparent (Fig. 6). Because of identical staining of similarly sized vesicles in the immediate vicinity of the dictyosome, it is concluded that the vesicles are derived from the Golgi system. They, too, like the ER membranes, are observed in the spindle interior along kinetochore fibers, but they appear to occur more randomly dispersed throughout the spindle interior.

DISCUSSION

The results show with new clarity the form and distribution of the NE-ER in the MA of a dividing plant cell. Through the use of OsFeCN staining that specifically delineates the NE-ER membrane system in barley, the dynamic processes of membrane transformation during mitosis have been reconstructed from micrographs. The results show, in particular, that elements of the NE-ER system aggregate at the spindle pole, surround the MA throughout mitosis, and invade the spindle interior, becoming intimately associated with the kinetochore microtubules. NE-ER membranes thus appear to aggregate and disperse, like the spindle fibers themselves to which they are closely associated. The temporal and spatial correlation between the membrane transformations and the structure and processes of the MA suggests that the NE-ER may be of considerable importance to the function of the motile elements during mitosis.

Before considering the function of these membranes, we must focus attention on certain aspects of their morphology and distribution in the cell. The most prominent structural

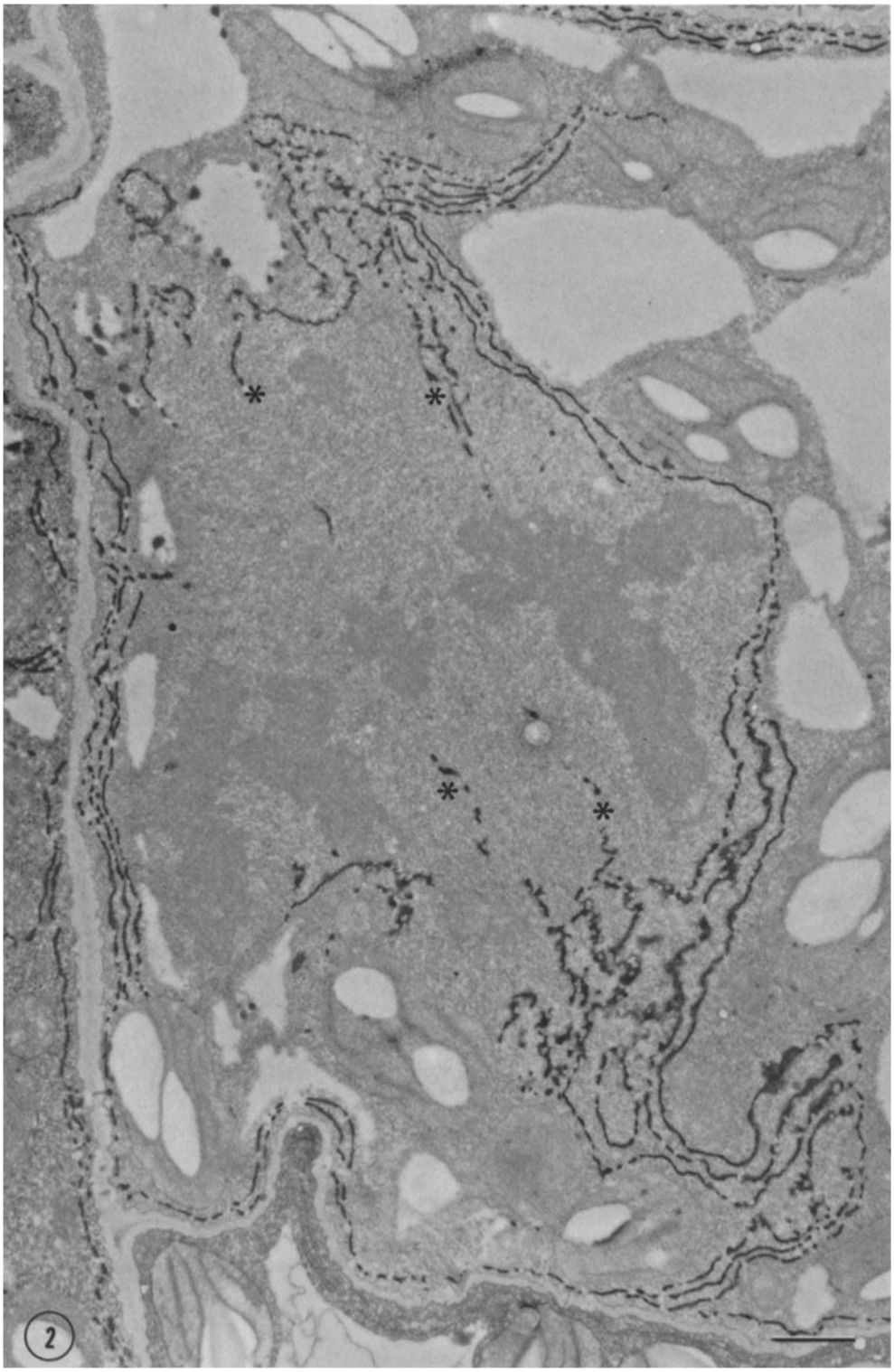
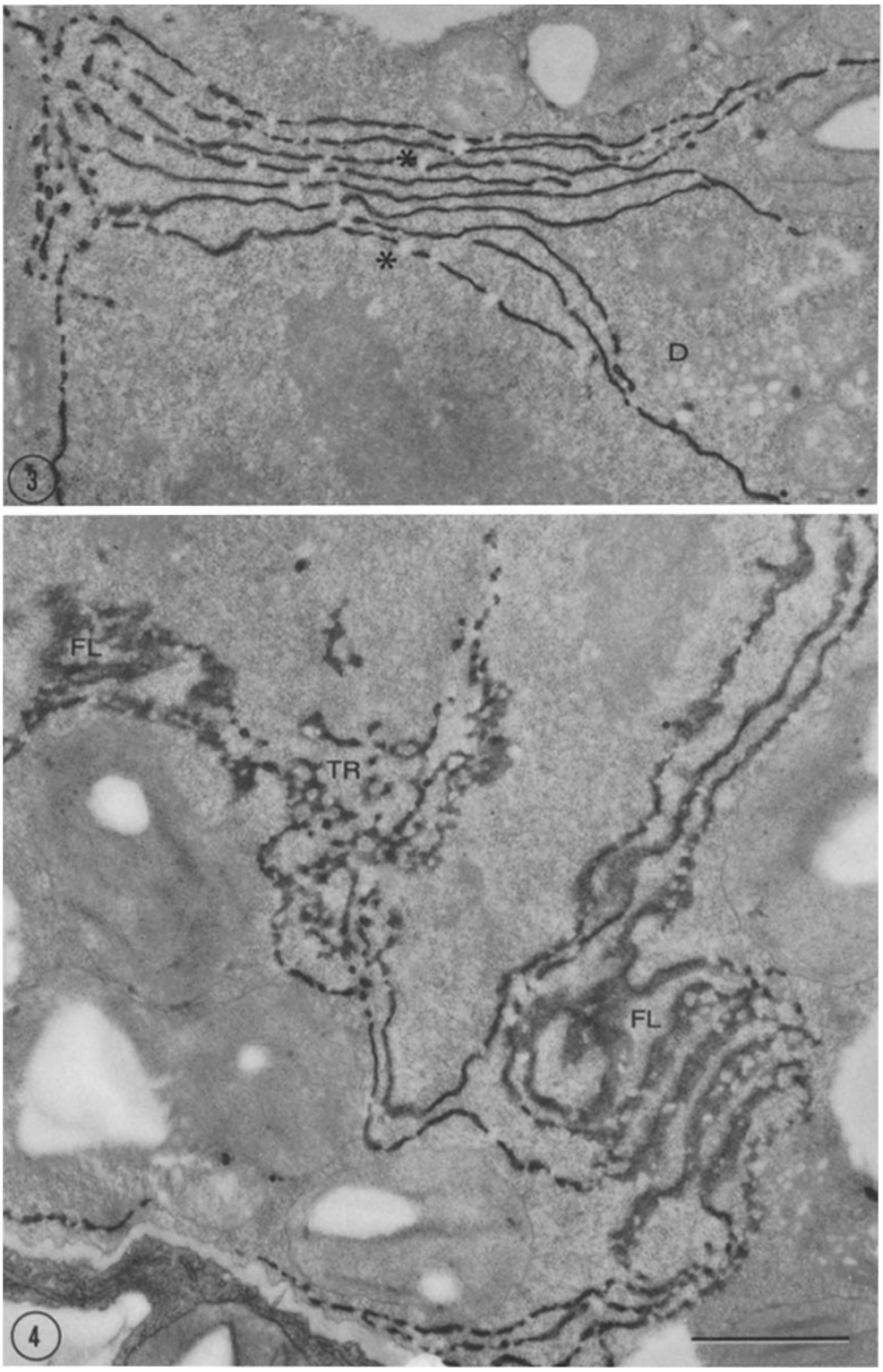


FIGURE 2 Metaphase in a mesophyll cell. The plane of section passes approximately parallel to the midplane of the metaphase plate. The spindle pole regions are characterized by aggregations of stained ER membranes, and extensions of these nearly surround the MA. Evident in this micrograph are stained membranes that extend into the spindle interior along kinetochore fibers (*). The kinetochore region in the upper right side of the MA is shown at higher magnification in Fig. 6. Bar, 1 μm. × 13,000.

transformation is the marked aggregation of NE-ER at the spindle poles. Some accumulation is observed soon after the breakdown of the NE, but the bulk occurs in the transition from metaphase to anaphase. Although it seems certain that the amount of membrane in the pole region increases, we have not been able to determine whether there is an increase in total membrane in the cell or whether there is simply a migration of the dispersed elements into the pole region. This question is currently under investigation.

While membranes have been observed previously in the



FIGURES 3 and 4 Enlarged views of the spindle pole at metaphase, from the same cell but different sections of the one shown in Fig. 2. Fig. 3: Aggregated elements of stained NE-ER characterize the pole region. Pores or fenestrations 40–100 nm in width occur extensively throughout the membrane system (*). The lack of electron density in the region of the pore suggests that ground cytoplasm has been excluded. *D*, dictyosomal vesicles. × 24,000. Fig. 4 shows the fenestrated lamellae (*FL*) sectioned tangentially (lower right and upper left) and reveals numerous pores in face view. The membrane in the center of the micrograph consists of a tubular reticulum (*TR*) and is continuous with the fenestrated elements. Bar, 1 μm. × 24,000.

poles of dividing plant cells (reference 13 for review), the OsFeCN staining has permitted us to demonstrate certain features of their ultrastructural detail that had not been noted. The staining shows that these membranes possess two different morphologies, tubular reticulum and fenestrated lamellae, that form a single continuous system. The fenestrated membrane

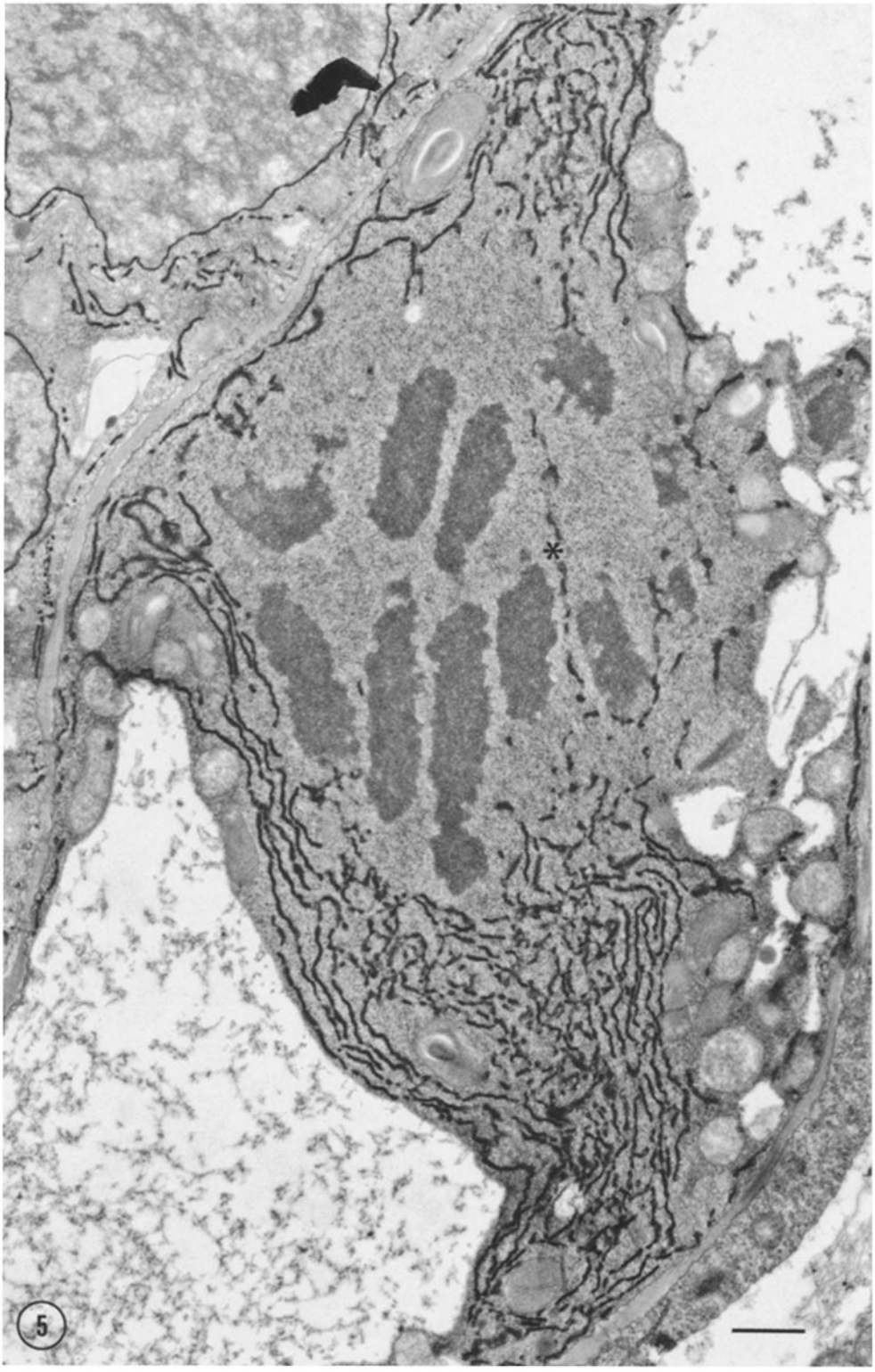
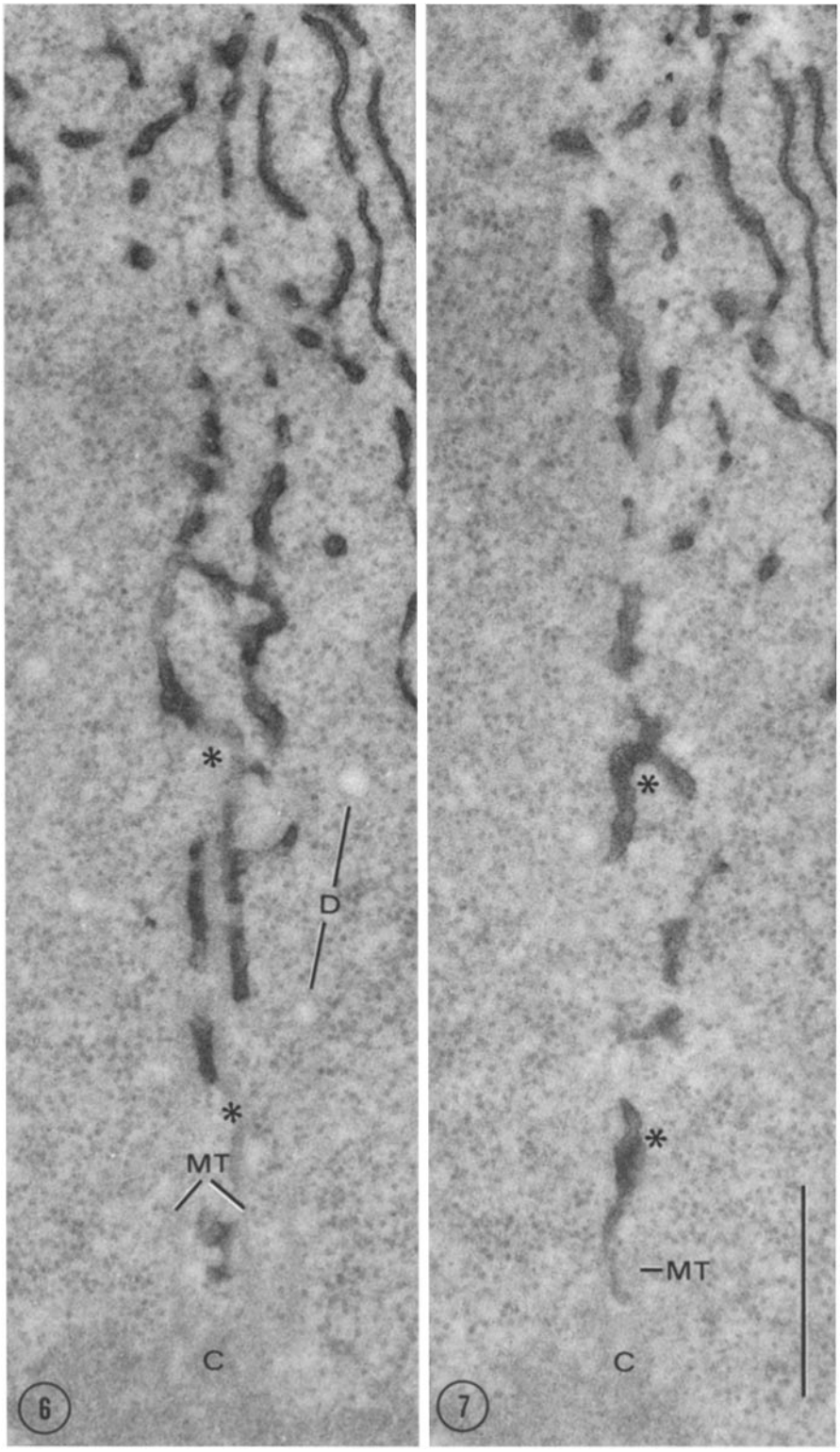


FIGURE 5 Early anaphase of a subsidiary cell. Membrane accumulation in the lower spindle pole is especially pronounced. The NE-ER appears to form a cap on the MA that excludes the cytoplasmic organelles from the spindle region. Some elements intrude into the MA that do not appear to be associated with kinetochores (*). Bar, 1 μm. × 11,000.

elements are particularly interesting because they are reminiscent of the fenestrated collar of the SR of muscle (5, 6, 17, 24). Because some of these MA membranes originate directly from the NE, it is possible that the fenestrations are derived from nuclear pores. It is noted, however, that nuclear pores are much more uniform and regular in outline than the fenestrations and contain distinctive structural differentiations that appear to be absent in the images produced herein.

Associated with the accumulation of NE-ER membranes at the poles is an extension of these elements along the sides of the MA. Images have been recorded in which the MA is virtually surrounded by membrane that appears to form a



FIGURES 6 and 7 Serial sections of the kinetochore region shown in Fig. 2. Microtubules, faintly visible, insert on the chromosomes (*C*) in the lower part of the micrographs. Reticulate tubular elements of ER extend inward along the microtubules almost to the chromosome itself. By comparing the two micrographs it becomes evident that the seemingly isolated ER segments are part of a single continuous membrane system. Notice how the two membrane systems singled out in Fig. 7 (*) effectively fill corresponding blank spaces between membrane elements in Fig. 6. Dictyosome vesicles (*D*) appear as unstained spheres. Bar, 0.5 μm. × 60,000.

boundary between the spindle and the rest of the cytoplasm. These examples resemble to some degree the "closed" spindles of lower organisms in which the NE does not break down (21).

One of the most novel and exciting aspects of the MA-associated membranes is their intrusion into the spindle interior specifically along kinetochore microtubules. This association has been observed from prometaphase through mid-anaphase. The tubular reticulate elements interdigitate between the ki-

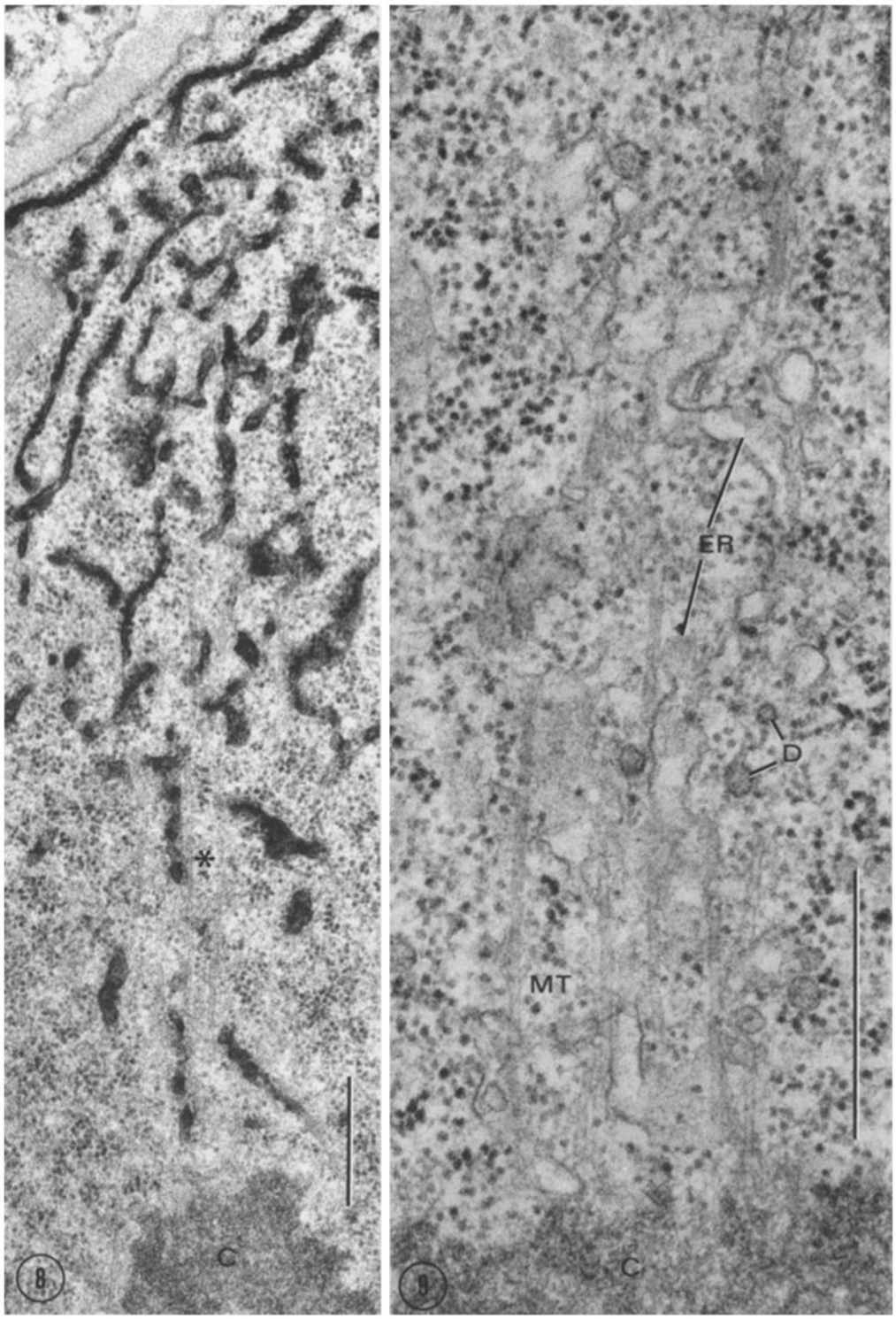


FIGURE 8 Kinetochore microtubule-membrane associations are revealed in material that has been postfixed with OsFeCN and subsequently stained in section with lead citrate. While membranes are even more sharply contrasted than with OsFeCN alone, the other cytoplasmic-spindle structures, especially microtubules (*MT*), become readily visible. Intermingling of membranes and microtubules, characteristic of kinetochore fiber bundles in barley, is demonstrated in this example from a cell in mid-anaphase. The asterisk shows an example of a membrane element positioned close to a microtubule. Chromosome, *C*. Bar, 0.5 μm. × 40,000.

FIGURE 9 The kinetochore region from a cell preserved by standard procedures of glutaraldehyde-osmium fixation. ER membranes, although not so distinct as with OsFeCN postfixation, are nevertheless evident and are seen to be closely associated with microtubules (*MT*) of the kinetochore region. With normal fixation procedures dictyosomal vesicles (*D*), depicted as small spherical bodies with dense granular contents, are also observed in the kinetochore region. Chromosome, *C*. × 80,000.

netochore tubules and are observed to extend from the pole to the point of chromosome attachment. The perceptive study of Porter and Machado (22), on potassium permanganate-fixed onion root tips, had much earlier noted penetrations of the ER into the spindle interior. Examination of their published micrographs strongly suggests that the membranes invade along kinetochore fibers. Unfortunately, the membrane-spindle relationship could not be explored more completely because the oxidative action of the permanganate fixative, we now know, had destroyed the microtubules.

The unique association between membranes and the MA, especially the kinetochore microtubules, indicates a structural differentiation that might underlie key physiological processes related to spindle formation and function. Foremost in our thinking is the idea that the ER, like the SR of muscle (4, 11), regulates the levels of free Ca^{++} in the spindle and thus controls the assembly and/or activation of the motile machinery (9, 10, 13, 15). The most obvious candidate for regulation by Ca^{++} is the microtubule that depolymerizes in the presence of elevated concentrations of the ion (26). But also to be considered are the presumptive actomyosin filaments which, by analogy to muscle, would be activated by increases in Ca^{++} concentration. The ER, like the SR, might contain a Ca^{++}-ATPase and be capable of alternately releasing and sequestering Ca^{++}, thus controlling ion concentration even in local regions, for example along kinetochore fibers and/or at the spindle pole.

There are a striking number of parallels emerging between the SR and the ER of dividing plant cells that cause us to look towards the SR as a paradigm in membrane structure and function, specifically in helping us develop our ideas about the ER and calcium regulation in the dividing cell. Beyond the fact that the SR is the ER of the muscle cell and that both SR and ER are associated with motile processes, there are more specific examples that support the similarity between these membrane systems. The first is that both SR and ER form a continuous membrane system throughout the cell. The fenestrated lamellae and tubular reticulum, especially at the poles of the MA, look remarkably similar to the fenestrated collar and tubular network SR observed overlying the "A" band of the muscle sarcomere (5, 6, 17, 24). Furthermore, in a recent study (7) microtubules in the sarcomere have been observed lying parallel to tubular SR elements, like the ER-kinetochore images reported herein. Although we have not observed structures similar to the terminal cisternae of the SR, the peripheral layer of ER in the cell cortex and the numerous ER-plasmodesmata connections insure the MA of numerous potential contacts with the plasmalemma and even with neighboring cells. Thus, it seems that the ER, like the SR, confines the motile machinery to an internal compartment within which the ionic milieu may be closely regulated. At the same time the ER, because of its continuity, creates channels through which signals may pass between the MA and the surrounding cytoplasm, the cell exterior, and neighboring cells.

A second major aspect of the similarity between SR and ER is the ability of both systems to react and stain positively with the OsFeCN reagent. A recent study argues that the enhanced staining by OsFeCN may be caused by the production of cyano-bridged iron-osmium complexes that are chelated by membrane proteins effective at sequestering metal ions (28). Calcium-binding proteins are obvious candidates, and it seems noteworthy in our work and that of Forbes et al. (5) that calcium must be present in the glutaraldehyde fixation in order for the subsequent OsFeCN staining reaction to occur. However, attempts to identify calcium in stained SR by energy dispersive x-ray analysis proved unsuccessful (5). Regardless of the reaction mechanism, that plant cell ER and muscle SR respond in an identical manner to OsFeCN suggests that these membranes share a common structure and/or composition.

A third area of confluence between SR and ER is their association with calcium and calcium-binding proteins. Using potassium antimonate, we show in the following paper that the spindle membranes, like muscle SR (25), contain deposits of bound calcium (29). Immunofluorescence studies of calmodulin also reveal that it is localized in the MA of rat kangaroo PtK2 and Chinese hamster ovary cells (27). While it is not known whether this calcium-dependent regulatory protein is associated with membranes, we note that its pattern of distribution is similar to that of the spindle-ER of barley. In addition, a spindle-associated Ca^{++}-ATPase has been reported by Petzelt and co-workers (16, 18–20). The enzyme is apparently membrane bound and fluctuates in activity in accord with the events of mitosis. Finally, the recent work by Silver et al. (23) reveals the presence of osmotically active membranes in mechanically isolated sea urchin MAs. These membranes show an ATP-stimulated uptake of ^{45}Ca from the medium. Taken together, these results are consistent with the view that the spindle-ER membranes contain enzymes similar to the Ca^{++}-ATPase of SR, and that they are able to accumulate Ca^{++} against a concentration gradient.

In addition to its postulated function in the regulation of calcium ion concentrations, it seems possible that the ER could play a structural role in the MA. Kinetochore microtubules are more stable in low temperature (1) and colchicine (2) than interpolar microtubules, and it seems reasonable that membranes, because of their intimate proximity, could contribute to this stability. Moreover, microtubules could cross-bridge to the membrane system (14) and use it as an anchor against which to generate force during chromosome motion.

It is evident, now, that membranes in the mitotic apparatus must be considered hand-in-hand with the motile elements if we are to elucidate chromosome motion and its control. So far, in these initial studies, our prior knowledge of the SR of muscle has proved valuable. In addition, with a deeper understanding of the motile proteins themselves and of possible calcium regulatory components such as calmodulin and the calcium ATPase, we should be able to develop fresh insights into the form and function of the MA.

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REFERENCES

1. Brinkley, B. R., and J. Cartwright, Jr. 1975. Cold-labile and cold-stable microtubules in the mitotic spindle of mammalian cells. *Ann. N. Y. Acad. Sci.* 253:428–439.
2. Brinkley, B. R., E. Stubblefield, and T. C. Hsu. 1967. The effects of Colcemid inhibition and reversal on the fine structure of the mitotic apparatus of Chinese hamster cells *in vitro*. *J. Ultrastruct. Res.* 19:1–18.
3. Clarke, M., and J. A. Spudich. 1977. Nonmuscle contractile proteins: the role of actin and myosin in cell motility and shape determination. *Annu. Rev. Biochem.* 46:797–822.
4. Endo, M. 1977. Calcium release from the sarcoplasmic reticulum. *Physiol. Rev.* 57:71–108.
5. Forbes, M. S., B. A. Plantholt, and N. Sperelakis. 1977. Cytochemical staining procedures selective for sarcotubular systems of muscle: modifications and applications. *J. Ultrastruct. Res.* 60:306–327.

6. Franzini-Armstrong, C. 1973. Membranous systems in muscle fibers. *In* The Structure and Function of Muscle. G. H. Bourne, editor. Academic Press, Inc., New York. 531–619. 2nd edition.
7. Goldstein, M. A., and M. L. Entman. 1979. Microtubules in mammalian heart muscle. *J. Cell Biol.* 80:183–195.
8. Hanzely, L., and O. A. Schjeide. 1973. Structural and functional aspects of the anastral mitotic spindle in *Allium sativum* root tip cells. *Cytobios.* 7:147–162.
9. Harris, P. 1975. The role of membranes in the organization of the mitotic apparatus. *Exp. Cell Res.* 94:409–425.
10. Harris, P. 1978. Triggers, trigger waves, and mitosis: a new model. *In* Monographs on Cell Biology. D. E. Buetow, I. L. Cameron, and G. M. Padilla, editors. Academic Press, Inc., New York. 75–104.
11. Hasselbach, W. 1972. The sarcoplasmic calcium pump. *In* Molecular Bioenergetics and Macromolecular Biochemistry. H. H. Weber, editor. Springer-Verlag, New York. 149–171.
12. Hepler, P. K. 1976. The blepharoplast of *Marsilea*: its *de novo* formation and spindle association. *J. Cell Sci.* 21:361–390.
13. Hepler, P. K. 1977. Membranes in the spindle apparatus: their possible role in the control of microtubule assembly. *In* Mechanisms and Control of Cell Division. T. L. Rost and E. M. Gifford, Jr., editors. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Pa. 212–232.
14. Hepler, P. K., J. R. McIntosh, and S. Cleland. 1970. Intermicrotubule bridges in mitotic spindle apparatus. *J. Cell Biol.* 45:438–444.
15. Hepler, P. K., and B. A. Palevitz. 1974. Microtubules and microfilaments. *Annu. Rev. Plant Physiol.* 25:309–362.
16. Mazia, D., C. Petzelt, R. O. Williams, and I. Meza. 1972. A Ca^{2+}-activated ATPase in the mitotic apparatus of the sea urchin egg (isolated by a new method). *Exp. Cell Res.* 70:325–332.
17. Peachey, L. D. 1965. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. *J. Cell Biol.* 25(3, Pt. 2): 209–231.
18. Petzelt, C. 1972. Ca^{2+}-activated ATPase during the cell cycle of the sea urchin *Strongylocentrotus purpuratus. Exp. Cell Res.* 70:333–339.
19. Petzelt, C. 1972. Further evidence that a Ca^{2+}-activated ATPase is connected with the cell cycle. *Exp. Cell Res.* 74:156–162.
20. Petzelt, C., and M. von Ledebur-Villiger. 1973. Ca^{2+}-stimulated ATPase during the early development of parthenogenetically activated eggs of the sea urchin *Paracentrotus lividus. Exp. Cell Res.* 81:87–94.
21. Pickett-Heaps, J. D. 1974. Plant microtubules. *In* Dynamic Aspects of Ultrastructure. A. W. Robards, editor. McGraw-Hill, New York. 219–255.
22. Porter, K. R., and R. D. Machado. 1960. Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip. *J. Biophys. Biochem. Cytol.* 7:167–180.
23. Silver, R. B., R. D. Cole, and W. Z. Cande. 1980. Isolation of mitotic apparatus containing vesicles with calcium sequestration activity. *Cell.* 19:505–516.
24. Somer, J. R., and R. A. Waugh. 1976. The ultrastructure of the mammalian cardiac muscle cell with special emphasis on the tubular membrane system. *Am. J. Pathol.* 82:191–203.
25. Suzuki, S., and H. Sugi. 1978. Ultrastructural and physiological studies on the longitudinal body wall muscle of *Dolabella auricularia*. II. Localization of intracellular calcium and its translocation during mechanical activity. *J. Cell Biol.* 79:467–478.
26. Weisenberg, R. C. 1972. Microtubule formation *in vitro* in solutions containing low calcium concentrations. *Science (Wash. D. C.).* 177:1104–1105.
27. Welsh, J. J., J. R. Dedman, B. R. Brinkley, and A. R. Means. 1979. Tubulin and calmodulin. Effects of microtubule and microfilament inhibitors on localization in the mitotic apparatus. *J. Cell Biol.* 81:624–634.
28. White, D. L., J. E. Mazurkiewicz, and R. J. Barrnett. 1979. A chemical mechanism for tissue staining by osmium tetroxide-ferrocyanide mixtures. *J. Histochem. Cytochem.* 27: 1084–1091.
29. Wick, S. M. and P. K. Hepler. 1980. Localization of Ca^{++}-containing antimonate precipitates during mitosis. *J. Cell Biol.* 86:500–513.