Effect of Digitonin on Cellular Division V. The distribution of microtubules

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Optical and ultrastructural investigations have shown that purified digitonin, a non-ionic, steroid-type saponin, dissociates the formation and function of the mitotic spindle from the phragmoplast (Olah 1963, 1965; Underbrink and Olah 1965, 1968).

The course of digitonin mitosis was described as similar to colchicine mitosis because it arrests the mitotic division at metaphase. However, during subsequent phases of digitonin mitosis, a vigorously functioning phragmoplast develops among the paralyzed and scattered chromosomes, resulting in a branched or amor- phously shaped cell plate and wall system which remains associated with the re- stituted tetraploid nucleus.

Our earlier studies using only KMnO_4 fixation were extended to include glutaraldehyde-osmium tetroxide fixation, in order to clarify whether the effect of digitonin is related to the formation and function of the microtubules which constitute the mitotic spindle and the phragmoplast.

Materials and methods

Cloves of *Allium sativum* were rooted in distilled water. Rootlets, approxi- mately 1 cm long, were treated for 15 hours in 0.25% and 0.5% purified digitonin solutions. Appropriate controls, placed in distilled water, accompanied the treat- ments. For electron microscopy, root tips were prefixed for 2 hours in 6% glutar- aldehyde in 0.15 M phosphate buffer (pH 7.4) containing 3 drops of 0.1 M CaCl_2 / 10 ml. The root tips were then rinsed for 30 minutes with the buffer and postfixed for 2 hours in 1% 0.15 M phosphate buffered OsO_4 at pH 7.4. Both fixations were carried out at room temperature. After 3-4 washes in distilled water, root tips were dehydrated in a graded methanol series, passed through propylene oxide, and embedded in Epon. Sections were cut with a diamond knife on a LKB ultra- microtome, placed on uncoated grids, stained with uranyl acetate and lead citrate, and examined with a RCA EMU-3C or Hitachi HU-11A electron microscope.

Results

In untreated cells, the microtubules showed their characteristic cortical,

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preprophase, chromosomal and continuous distribution during the various stages of the mitotic cycle.

Examination of interphase cells in treated root tips did not reveal any visible effects. The microtubules, as in untreated cells, showed a typical cortical disposition (Figs. 10, 13). The first indication of the effect of digitonin was marked by the apparent absence of microtubules representing a preprophase band. Microtubules were also absent at late prophase (Fig. 1) and meta-anaphase (Fig. 2). In some cells, however, short fragmented microtubules were found, either associated with the kinetochores, or scattered in the cytoplasm without any distinct orientation.

In marked contrast to earlier stages of digitonin mitosis, phragmoplast microtubules were frequently observed at telophase or early interkinesis. These microtubules were always found in association with elements of the forming cell plate segments (Figs. 3-4). Moreover, as far as geometrical conditions of the forming cell plates permitted, these microtubules always tended to orient perpendicular to the plane of the cell plate segments. Figs. 3 and 4 further illustrate that these microtubules typically terminated at the level of the fusing vesicles of the cell plates.

Since the forming cell plate segments were often randomly scattered or branched, a corresponding haphazard orientation of the microtubular bundles was also apparent. As illustrated in Fig. 3 (upper arrows), such bundles often diverged from focal areas. These "spindlets" appeared to be functional, since among their diverging microtubules, some vesicles were always present. The number of vesicles increased toward the diverging ends, and at the terminal region, they eventually fused to form a portion of the aborted cell plate. Around more developed cell wall segments, microtubules were generally absent (Fig. 3, bottom).

In the forming cell plate regions, two kinds of vesicles were predominant; small electron-dense, single membrane-bound vesicles and somewhat larger sized (300-400 Å) electron-light vesicles. The latter, rather irregularly shaped vesicles, were also single membrane-bound, and were probably derived from dictyosomes situated in the vicinity of the forming cell plate (Fig. 3).

Cells in interphase frequently contained tightly packed bundles of parallelly associated microtubules in the vicinity of the nucleus or formed cell wall segments (Figs. 5-7). The orientation of such bundles also seemed to be haphazard. As illustrated in Fig. 6, they were often sandwiched between the induced cell wall portions and restituted nuclear areas. The number of microtubules per bundle varied greatly (6-160). Adjacent microtubules of such bundles never contacted each other and revealed a minimal center-to-center spacing of about 370 to 400 Å (Figs. 5-7). Occasionally, the orientation of these bundles revealed slight changes giving the impression of a low degree of twisting (Figs. 6, left arrow; and 7), with few exceptions, vesicles were rare between these microtubules.

Comparison of the restituted stages of the nuclei with the maturation stages of the induced cell wall systems indicated that some of these cells were in advanced interphase. Since, in treated cells, cell wall formation may be delayed (Olah, 1965), the microtubules in these cells probably appeared at the period of the mitotic cycle when cortical microtubules formed along the cross wall (Figs. 18-19). In treated cells, such post-mitotic microtubules were oriented parallel to one another

Figs. 1-2. 1, late prophase in treated cell (0.25% digitonin). Microtubules absent throughout the cell. Chromosomes visible as densely stained bodies. Nucleolus (NU) still present as loosely arranged granular mass. $\times 8,600$. 2, portion of arrested metaphase (0.5% digitonin) showing absence of spindle microtubules. Profiles of endoplasmic reticulum (ER) evident among chromosomes. K, kinetochore. $\times 20,000$.

Fig. 3. Portion of developing cell plate between two restituted nuclear regions. Vesicles, which appear to have arisen from the dictyosomes (D), are aggregating in large numbers between clusters of randomly oriented microtubules (arrows). Microtubules located close to cell plate region show perpendicular orientation of coalescing vesicles. 0.5% digitonin. $\times 40,000$.

Figs. 4-5. 4, cell plate in later stage of development (0.5% digitonin). Some vesicles have undergone considerable fusion. Microtubules show a perpendicular orientation to coalescing vesicles. 5, large number of randomly oriented bundles of microtubules (arrows) adjacent to restituted nucleus (N) and cell wall (CW). 0.25% digitonin. $\times 60,000$.

Figs. 6-8. 6, disposition of microtubules (arrows) relative to cell wall material (CW). 0.25% digitonin. $\times 25,000$. 7, higher magnification of a part of the cell wall material and associated microtubules shown in Fig. 6. Microtubules not in contact with one another and some show close association with the plasmalemma of the cell wall (CW). $\times 34,000$. 8, cell wall material (CW) within nuclear invagination. Microtubules evident in both transverse and longitudinal profiles (arrows). 0.5% digitonin. $\times 26,000$.

Figs. 9-13. 9, cortical microtubules (arrows) located subjacent to plasmalemma of cell wall system (CW). Arrow (labeled "F") indicating fibrils within cell wall. (D), dictyosome. 0.25% digitonin. $\times 23,500$. 10, portion of lateral cell wall (CW) with adjacent cortical microtubules. 0.5% digitonin. $\times 54,000$. 11, portion of digitonin-induced cell wall system (CW) attached to mother cell wall. Arrows indicate cortical microtubules in neighbouring cell. 0.25% digitonin. $\times 34,000$. 12, portion of cell wall material (CW) and adjacent cortical microtubules. 0.25% digitonin. $\times 20,500$. 13, portion of grazing section of one cell including part of the cell wall (CW) and microtubules within the cell cortex. 0.5% digitonin. $\times 51,500$.

and generally located adjacent to the plasmalemma of the cell wall system (Figs. 6–7 and 9). In some cells, however, it was difficult to discriminate between phragmoplast and cortical microtubules (Figs. 5 and 8). The groups visible between the maturing cell wall portions may have been involved in cell plate formation, since some electron-light vesicles are still discernible at their diversing ends. On the other hand, the microtubules located adjacent to the plasmalemma of the cell wall systems probably represent cortical microtubules. Both phragmoplast and cortical microtubules showed slight undulations along their lengths (Figs. 3, 5 and 13).

Direct evidence for the appearance of cortical microtubules along the aborted and matured cell wall is seen in Fig. 9 (arrows). Microfibrils, sometimes demonstrating parallel orientation with the cortical microtubules, may also be traced (F-labelled arrow). Cortical microtubules running parallel with the lateral maternal wall were also found in cells after digitonin mitosis (Figs. 10 and 12). Further evidence, that digitonin did not interfere with the formation of cortical microtubules, is shown in Fig. 11. In such cases, cortical microtubules, were absent in the dividing cell (Note CW, left), while the adjacent interphase cell still showed the presence of typical cortical microtubules (arrows).

Grazing sections of areas close to the plasmalemma also revealed the presence of cortical microtubules (Fig. 13). The abnormally thickened portion of the cell wall indicated in this case that the cell completed a digitonin mitosis. Underbrink and Olah (1965) frequently observed such thickenings of the maternal wall system in treated cells.

Figs. 15, 17 and 19 represent interpretive drawings of our observations on digitonin-treated cells. Early telophase is shown in Fig. 15; transition between late telophase and early interphase is represented by Fig. 17; while Fig. 19 depicts a restituted interphase cell with cortical microtubules. For comparison, corresponding stages showing phragmoplast and cell plate formation in untreated cells are depicted in Figs. 14, 16 and 18.

Discussion

From evidence of optical, ultrastructural and *in vivo* studies (Timberlake 1900; Wada 1932, 1936, 1938 a, b, 1939 a, b, 1940, 1941, 1944, 1950, 1952 a, b, 1969, 1970; Wada and Izutsu 1961; Wada and Hanaoka 1964; Bajer 1957, 1958 a, b, 1965 a, b, 1967, 1968 a, b; Bajer and Allen 1966 a, b; Bajer and Mole-Bajer 1954, 1956, 1961; Mole-Bajer 1965, 1967, 1969; Inoue 1953, 1964; Inoue and Bajer 1961; Inoue and Sato 1967; Sato 1958, 1959, 1960; Esau and Gill 1965; Cronshaw and Esau 1968; Hepler and Jackson 1968, 1969; Pickett-Heaps 1969; Pickett-Heaps and Fowke 1968; Pickett-Heaps and Northcote 1966 a, b; Olah 1969; Olah and Hanzely 1969), a new picture emerged separating two microtubular systems in higher plants, namely, the mitotic and the phragmoplast spindles.

It is now well recognized that one of the first steps in the geometric foundation of mitosis is the formation of the pole-to-pole (continuous) microtubules, or the often termed "central spindle". The microbeam irradiation experiments of Campbell and Inoue (1965) and Inoue and Sato (1967) demonstrated that the polar

cell centers and the kinetochores were the orienting centers of the mitotic spindle microtubules. The disposition of the phragmoplast microtubules, in turn, was governed by a third orienting center, i.e., the forming cell plate.

In order to gain more insight into the nature of the selective effect of digitonin, we must consider the origin and functional relationship of these separate microtubular systems and the nature of their organizing centers. In higher plants, however, the lack of a specialized morphological organization of the polar areas is a disturbing fact. One may assume that some of the membraneous elements concentrated in these areas (Porter and Machado 1960; Avers 1963) are endowed with a nucleating function.

Since in meristematic cells the mitotic and phragmoplast spindles overlap each other, the role of the continuous microtubules, relative to the phragmoplast, is debatable. In this regard, Bajer (1968 b) stated that these microtubules still persisted after the initiation of cell plate formation. Hepler and Jackson (1968), however, observed only a few remaining spindle microtubules at the initial stages of cell plate formation. We also supported the assumption that all spindle microtubules disappear before or during the polymerization of the phragmoplast microtubules (Olah 1969; Olah and Hanzely 1969). If this would not be correct, the central portion of the spindle would contain two different elements, one formed at prophase and the other at telophase.

In treated cells, many forming plate segments were densely associated with microtubules but they always terminated at the level of the plate (Fig. 4). Interdigitations or clusterings of the type found by Hepler and Jackson (1968) were not observed. Such spindlets were functional, but unable to achieve normal geometry (Fig. 3). Their function seemed to be involved with guidance of the vesicles to their destination. Since continuous microtubules were never observed in treated cells, it is obvious that they were not essential for plate formation. Therefore, our concept about the microtubular organization of the phragmoplast (Olah 1969; Olah and Hanzely 1969) is in agreement with that of Ledbetter (1967), who visualized the phragmoplast to be composed of two microtubular half-spindles (Fig. 16).

Analysis of the unique patterns of phragmoplast development in digitonin-treated cells indicates that nucleation of the phragmoplast microtubules may be initiated at the forming plate. This would imply that cell plate elements appear first, and that microtubular association is a secondary phenomenon. On the other hand, it is also apparent that phragmoplast microtubules seem to guide vesicles to the plate. Thus, plate formation appears to depend on the pre-existence of oriented microtubules. To understand this circuitous reasoning, it may be assumed that some specific elements in the phragmoplast (vesicular or membraneous) are able to initiate nucleation. These elements may first appear and orient the microtubules, which, in turn, almost simultaneously start to guide vesicles to their destination. In *Allium*, a heterogeneous population of membraneous and vesicular elements were indeed noted in the area of the forming cell plate by Porter and Machado (1960). Becker (1934), using vital stains, recognized two types of granules, a smaller one which participated in plate formation, and a larger one (droplets) from which the plate formed. Bajer (1965a) assumed that small granules

in the interzonal region and in the phragmoplast arose *in situ*, and that a difference existed between these small granules and the plate-forming droplets. In *Zea* and *Spinacia*, the array of small electron-dense vesicles, which appears prior to the large ones (Whaley and Mollenhauer 1963; Whaley *et al.* 1966), may carry specific information related to microtubular disposition. Although Frey-Wyssling *et al.* (1964), as well as some other investigators, have assumed that all vesicles in the cell plate region were dictyosome-derived, it may be that some of the vesicles, endowed with a nucleating function, are of different origin.

In the branching cell plate system of treated cells, segments of markedly different maturation stages were randomly disposed. Segments of the earliest stages of maturation were always associated with a high number of microtubules (Figs. 3, 4, 15 and 17), while mature segments lacked an association with phragmoplast microtubules. Instead, a new element, namely the cortical microtubule, appeared adjacent to the plasmalemma of the cell wall system (Figs. 9 and 17). Since mature segments were no longer associated with microtubules, it may be assumed that depolymerization of microtubules is a function of the vesicle membranes, which fuse and form the new plasmalemma. Such a morphogenetic force may successively be activated during maturation of the plate.

The dissociating effect of digitonin was described in a series of earlier papers (Olah 1963, 1965; Underbrink and Olah 1963, 1965; Olah and Bozzola 1972). It was also pointed out (Olah 1969) that in coenocytial endosperm and certain pollen mother cells (PMCs), a multispindle system forms due to the total chronological and spatial dissociation of the mitotic spindle from the phragmoplast spindle. While the dissociative effect of digitonin is based on a selective impairment or elimination of microtubules, the microtubular organelles of coenocytial endosperm and PMCs are separated from each other by a mechanism which simply delays the formation of the phragmoplast spindle by one or more mitotic cycles. The simplest case involves the PMC, in which cell plate formation is delayed by one mitotic cycle. During the second telophase in this four nucleate coenocytium, four phragmoplast spindles are formed (Fig. 20). Two of these connect sister nuclei and overlap the areas in which mitotic spindles were located. Conversely, the other two, linking non-sister nuclei, are formed in a cytoplasmic area which is void of mitotic spindle elements. Accordingly, the terms "mixed" and "pure" phragmoplast spindle may be applied to these systems (Note Fig. 20 solid and dotted lines). In "nuclear" endosperm (Fig. 22), cell plate formation may be delayed by several nuclear divisions. The ratio between mixed and pure phragmoplast spindles depends on several factors. This, however, is difficult to analyze unless a certain geometrical order is assumed. Initially, there must be an assumed critical distance (Note Figs. 21, diagonal lines; and 22, A and B). In turn, nuclei remaining at a critical distance must then form rows and consequently layers. In such a system, the ratio between the number of mixed and pure spindles would depend on whether an overlapping or alternating position existed between the nuclei forming rows (of the superimposed layers). In a monolayer in which the nuclei form parallel rows in register, the ratio would be 1:3. If the rows were not in register, and the nuclei were in alternating positions, the ratio would be 1:5. In the simplest multilayered

system, all nuclei would be in register (like atoms in a NaCl crystal) with a ratio of 1:5 (Fig. 23), but if the rows alternated the ratio would be 1:7 (Fig. 24). In a multilayered system with alternating nuclei in each of the layers as well as with an alternating position of the layers, the ratio would be 1:11. The logical conclusion is that the continuous spindle microtubules are not essential, and most probably disappear prior to the formation of the phragmoplast microtubules (Fig. 14).

The apparent nuclear interaction, resulting in such multispindle systems, de-

Figs. 14-24. 14, initial stage of cell plate formation. All microtubules terminate at the level of forming plate. 15, similar stage in treated cell. 16, advanced stage of plate formation in telophase. Based on Ledbetter's concept (1967). 17, late telophase in treated cell. Note haphazard orientation of microtubular bundles. 18, interphase. Note reappearance of cortical microtubules along all walls. Postmitotic cell elongation omitted. 19, interphase in treated cell. Note cortical microtubules along aborted cross wall. Postmitotic cell elongation omitted. 20, PMC with four phragmoplast spindles formed during second division. Solid lines represent phragmoplast spindles which overlap mitotic spindles. 21, PMC found in *Dodecatheon* sp. Fifth phragmoplast spindle formed between nuclei within the critical distance. 22, Multispindle system in *Sequoia* endosperm. Nuclei A and B are beyond critical distance. Spindle is absent. Redrawn from Bucholz (1939). 23, nucleus with six spindles representing building unit of multispindle system in a coenocytium with nuclei located similar to atoms of table salt. 24, building unit in a coenocytium similar to *Sequoia* endosperm.

serves further attention. Van Wisselingh (1909) found, in centrifuged *Spirogyra*, that the position of the nucleus prior to dislocation may determine the position of the cross wall. From his results, it seems to us that only those nuclei exert this influence which are in the G₂ period. The nature of this morphogenetic force is unknown. In digitonin-treated cells, the constant association of the phragmoplast complex, with the restituting nucleus, indicates that the initiation of the phragmoplast may be under nuclear influence (Olah and Bozzola 1972). Thus, one may speculate that a morphogenetic message emanates from the nucleus and that the disposition of some specific elements is under the control of the nucleus. These elements, in turn, would represent the nucleating center(s) that orient the phragmoplast microtubules.

Digitonin, as a selective dispersing agent, is used for the liberation of the "stabilized" mitotic apparatus (Mazia 1960) as well as cilia (Child and Mazia 1956). Haglund and Lovtrup (1965) found that digitonin alters the permeability of the cortical surface membrane in living *Rana* eggs. Thus, its effect on processes controlled by a concentration equilibrium may be visualized as an interference with the functional role of the orienting center. Digitonin may also disrupt polymers or prevent polymerization by complexing with the corresponding monomer(s). Polymerization of microtubules and formation of the digitonin micelle are both thermodynamically controlled processes. Thus, mutual interference is conceivable. If the nature of the self-assembling subunits were such that they were able to take up mutual positions, then a light conformational alteration in the steric configuration induced by digitonin may impair such subunit association.

The postulated selective effect on orienting centers may be explained by the assumption that the kinetochore (as a precisely localized nucleating center) is more sensitive to environmental changes, induced by digitonin, than the forming cell plate. Alternatively, digitonin may complex with the monomer unit which is involved in mitotic spindle formation, but not with the monomer unit of cortical and phragmoplast microtubules. This would imply the existence of two monomers, one active in a prophase environment and the other in a post-metaphase environment. Hubbard (1954, 1958) found that the digitonin micelle was able to undergo selective complexing with proteins, since it complexed with opsin (protein moiety of the visual pigment) but not with ovalbumin. It is pertinent to note that digitonin is also able to induce profound architectural alterations of polymerized structures, as attested the appearance of oversized tubular elements in treated *Allium* cells (Hanzely and Olah 1970).

Since both assumptions (selective effect on nucleating centers and selective complexing) imply that microtubules have a different chemical and/or architectural makeup, we must review the topic related to the monomer and its assembly into microtubules. Mazia and Dan (1952) isolated from the mitotic apparatus and flagella proteins with a sedimentation constant of 4. Zimmerman (1960) isolated a 3.7 S and 8.6 S fraction, while Dirksen (1964) demonstrated the presence of 14 S and 21 S proteins. Kane (1967) isolated a 22 S protein as the major component of the spindle. Sakai (1966), using dithiodipropyl alcohol, obtained 3.5 S, 13 S and 21–22 S proteins. In turn, sulfite treatments cleaved the 3.5 S and 13 S aggregates

into 2.5 S subunits with a molecular weight of 34,700. Stephens (1967) further showed that the 22 S protein yielded hexamers, tetramers and dimers with a molecular weight of 105,000–120,000. In another investigation, Stephens (1968) re-associated solubilized 2.9 S subunits, isolated from flagellar outer doublets, into tubule-like structures.

The number of microtubular subunits or protofilaments was reported to be 13 by Ledbetter and Porter (1964). However, this may be subject to variation, since Bertolini *et al.* (1970) have provided evidence, that in ganglion cells, two categories of microtubules exist, i.e., one with 9 and the other with 10 subunits. Behnke and Forer (1967) found that microtubules of crane fly spermatids and rat sperms responded differently during pepsin and temperature treatment. Based on their studies, they concluded that there were four distinct classes of microtubules. Furthermore, three differently sized microtubules were found by Moor (1967) in freeze-etched preparations of yeast.

The selective effect of *Vinca* alkaloids (Mesquita and Mangenot 1967, Bensch and Malawista 1968, 1969), which, in interphase cells, sequester microtubular proteins and induce polymerization of tubular crystals, provides another interesting aspect concerning the microtubular monomer. Marantz *et al.* (1969) vinblastine precipitated the colchicine binding microtubular protein. An interconversion of neurofilaments (assumed protofilaments) into induced tubular crystals was observed by Wisniewski *et al.* (1968) who assumed that the protofilaments were ribbon shaped and built from dimer units.

While a number of discrepancies still exist relative to the microtubular monomer, the protein which complexes colchicine seems to be a dimer. Taylor (1965) and Borisy and Taylor (1967 a, b) isolated a 6 S protein- H^3 labelled colchicine complex from a broad variety of animal tissues, while Shelanski and Taylor (1967, 1968) isolated the same complex from the central pair tubules of flagella. The paired outer ciliary microtubules also yielded a 6 S complex (Renaud *et al.* 1968). Furthermore, these microtubular dimers were shown to possess two binding sites for GTP, and one for colchicine (Stephens *et al.* 1967; Weisenberg *et al.* 1968).

Adelman *et al.* (1968) visualized the subunits of all microtubules as closely related 6 S proteins or tubulins with a molecular weight of 120,000. Under proper conditions, these tubulins aggregate by self-assembly, while a spatial distribution of the binding sites determines the geometry of the polymer. Conversely, subtle differences may be visualized as conformational alterations. Moreover, it is clear that such alterations in the secondary or tertiary structure would change the ability of the subunit to establish the expected geometry. Whether mitotic agents or an externally changed environment (cellular responses) can induce such conformational changes remains to be answered. According to Monod's classical concept, small molecules (activators or inhibitors), attached to a protein, induce conformational changes. Relative to this, Adelman *et al.* (1968) suggested that the polymerizable or non-polymerizable state of the microtubular subunit may depend upon the attachment of GTP. Thus, one may speculate that the selective effect of digitonin indicates the presence of two categories of tubulins in the plant cell. The first group complexes with digitonin while the second does not. Reactivity

would then depend on subtle but well-defined structural differences. Subunits forming spindle microtubules would belong to the first category. Since we did not find a preprophase band in treated cells, we are inclined to include the microtubules of this band in the same category. Phragmoplast and cortical microtubules, on the other hand, would belong to the second category since they are post-metaphase microtubules and apparently do not complex with digitonin.

Caffeine and its derivatives were also shown to be capable of dissociating the mitotic spindle from the phragmoplast. This effect, however, appears to be just the opposite to that of digitonin. Lopez-Saez *et al.* (1966) and Risueno *et al.* (1968) have shown that caffeine prohibited the coalescence of the plate-forming vesicles, although their fixative did not permit the behavioral study of the microtubules. Thus, caffeine apparently suppresses the phragmoplast, while the formation of the mitotic spindle is not affected. Wada (1971), based on our earlier publication, supposed that digitonin disturbed the orientation of spindle microtubules, because in the absence of a parallel orientation, chromosomal movement was either partially or entirely blocked. Indeed, such an effect was found by Hepler and Jackson (1969) using isopropyl-N-phenyl carbamate. Digitonin, however, effects microtubules more specifically and selectively. Further investigations will show whether the selective effect of digitonin is indeed a unique and rare phenomenon. Along with digitonin, the effects of caffeine and the *Vinca* alkaloids indicate that the selective response of microtubule proteins is not entirely unique but may occur more frequently than previously supposed.

Summary

Ultrastructural studies of glutaraldehyde-osmium tetroxide fixed root tip cells of *Allium sativum* revealed that digitonin prevented the formation of microtubules of the mitotic spindle. In telophase, however, a large number of microtubules always formed. These phragmoplast microtubules were functional in the development of a randomly oriented cell plate. Consequently, the restituted tetraploid nucleus was always associated with a highly branched wall system. A comparative study of treated and untreated cells has indicated that the "mitotic spindle" and the "phragmoplast spindle" are two distinct and functionally independent microtubular organelles. Furthermore, it is suggested that the microtubular elements present in these two organelles are not entirely identical in their chemical or architectural makeup.

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