Microtubule-determined plastid distribution during microsporogenesis in Lilium longiflorum

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Summary

The relationship between organelle distribution and the cytoskeleton was examined during microsporogenesis in Lilium longiflorum. The distribution pattern of plastid and mitochondrial nucleoids was followed by fluorescence microscopy after staining with 4',6-diamidino-2-phenylindole (DAPI). Although the plastid nucleoids gradually enlarged during prophase I, by anaphase I of meiosis they were randomly distributed in the cytoplasm of each microsporocyte. At telophase I the plastid nucleoids were aggregated in the equatorial region of the cell. After entering prophase II the plastid nucleoids were randomly distributed in the cytoplasm, and at telophase II they had reaggregated to the equators of the two cells. After the completion of meiosis they were located at the two poles of each young microspore. This distinct cell polarity of plastid nucleoids was preserved in isolated protoplasts. In all cells where the distribution of plastid nucleoids was non-random, the nucleoids were invariably situated furthest away from the interphase and telophase nuclei. However, the distribution of mitochondrial nucleoids throughout meiotic division showed little cell polarity.

Analysis of the microtubule and actin cytoskeletons during microsporogenesis revealed that the microtubules radiated out from the cell nuclei only at the stages when the distribution of plastids showed polarity, whereas the actin filaments were usually randomly oriented throughout the cytoplasm, independent of the plastid arrangement and of the organization of microtubule cytoskeleton. The radiating microtubules seemed to exclude the plastids from around the cell nuclei. Treatment of cultured pollen tetrads with colchicine disrupted the plastid polarity, probably by depolymerizing the radiating microtubules, resulting in a random distribution of the plastid nucleoids. Treatment with cytochalasin B, however, had no effect on the arrangement of plastids.

These results demonstrate that microtubules function in the movement and distribution of plastids in male reproductive cells of higher plants. Further, it is assumed that the system of radiating microtubules that controls the distribution of plastids during male meiosis is also involved in the subsequent formation of male gametes, which are deficient in plastids in many angiosperm plants, including this lily.

Key words: Lilium, meiosis, microtubule cytoskeleton, plastid, polarity.

Introduction

The structure of the microtubule and actin cytoskeletons during male meiosis in higher plants has been studied in a number of species by immunocytochemistry and rhodamine–phalloidin staining. Van Lammeren et al. (1985), Sheldon and Dickinson (1986), Hogan (1987) and Traas et al. (1989) used immunofluorescence to show the pattern of microtubules, while Sheldon and Hawes (1988), Traas et al. (1989) and Van Lammeren et al. (1989) used fluorescent-dye-labelled phallotoxins to visualize the structure of actin filaments (F-actin). These studies concentrated mainly on the roles of the two cytoskeletons in division processes, in particular their function in nuclear division and cytokinesis through formation of spindles and phragmoplasts. However, during microsporogenesis the microtubules and actin filaments are thought to be involved in other functions, including cytoplasmic transport, positioning of the nucleus and organelle distribution.

The cytoplasmic events occurring during male meiosis have largely been demonstrated by electron microscopy (Maruyama, 1968; Dickinson and Heslop-Harrison, 1970; Dickinson, 1981; Bird et al. 1983). Although the behavior of organelles is not the same in all higher plants, the appearance of cell polarity in organelle distribution has been reported in several cases. In Lilium, Dickinson (1981) showed that plastids were located at the poles of each microspore, whereas in Cosmos mitochondria were associated with the nucleus at the same tetrad stage (Dickinson and Potter, 1979). Such cell polarity in the distribution of plastids and mitochondria during microsporogenesis is also seen in other species, including gymnosperms and ferns (Willemse, 1972; Wolniak, 1976; Bednara et al. 1986; Rodkiewicz et al. 1986). These studies also suggest that the distribution of organelles might be controlled by the...
cytoskeleton. However, the evidence is insufficient to conclude that there is a definite relationship between the two.

It has generally been difficult to observe these organelles by light microscopy. However, fluorescence microscopy after staining with 4′-6-diamidino-2-phenylindole (DAPI), a DNA-specific fluorochrome, enables small amounts of DNA such as chloroplast DNA to be detected (Coleman, 1978, 1979; James and Jope, 1978; Kuroiwa et al., 1981). Using DAPI staining, Miyamura et al. (1987) showed the presence of plastid and mitochondrial nucleoids in cells during microsporogenesis. This technique is superior to electron microscopy in visualizing the distribution of organelles such as plastids and mitochondria throughout the cell.

I have therefore used fluorescence microscopy, with three kinds of fluorescent probes, to examine the role of the microtubule and actin cytoskeletons in organelle movement and distribution in lily microsporocytes and microspores. These cells have many advantages for such studies. First, the developmental process during microsporogenesis is highly synchronized and the successive stages are closely correlated with bud length (Ito and Stern, 1967; Tanaka et al., 1979). The cells are also large, and protoplasts can be obtained at each stage (Ito, 1973; Tanaka et al., 1987). Further, the organization of the microtubule and actin cytoskeletons during microsporogenesis has been described previously in Lilium by other investigators (Dickinson and Sheldon, 1984; Sheldon and Dickinson, 1985; Sheldon and Hawes, 1986).

I show that microtubules radiating from the cell nuclei, which were first reported in Lilium by Dickinson and Sheldon (1984), and which commonly appear during male meiosis in other higher plants (Van Lammeren et al., 1985; Hogan, 1987; Traas et al., 1989), play an important role in plastid arrangement during male meiosis. The significance of the specific distribution of plastids is discussed further with respect to male gamete formation after meiosis.

Materials and methods

Plant material

The experimental material was Lilium longiflorum cv. 'Georgia', grown in a greenhouse. A correlation between the successive stages of microsporogenesis and bud length in this lily has previously been reported (Ito and Stern, 1967; Tanaka et al., 1979), but it varies slightly with culture conditions. Under greenhouse conditions, meiosis begins when the bud is 13 mm in length and is completed by the time the bud reaches 27 mm (pollen tetrads). In this study, premeiotic cells (11 mm buds), microsporocytes taken during meiotic division (13–27 mm buds), pollen tetrads (27–28 mm buds) and uninucleate microspores just after the liberation from tetrads (28 mm buds) were isolated from anthers of each bud. Each anther was cut open at one end with a sharp forceps, and cells were extruded by gentle squeezing from the end distal to the cut. In the anthers of buds 27–28 mm in length, at least two kinds of pollen tetrads can be recognized under an ordinary microscope; one is the early–mid tetrad in which the formation of pollen exine consisting of sporopollenin does not occur, the other is the late tetrad in which exine formation has begun.

Protoplast isolation

The microsporocytes extruded during meiotic division and pollen tetrads at the early–mid stage were suspended directly in enzyme solution, which contained 1% (w/v) Macerozyme R-10 (Yakult Honsha Co., Ltd, Tokyo, Japan), 1% (w/v) Cellulase Onozuka R-10 (Yakult), 0.1% (w/v) pectolylase Y23 (Seikin Pharmaceutical Co., Ltd, Tokyo, Japan), 0.1% (w/v) Zymolyase (Seikagaku Kogyo Co., Ltd, Tokyo, Japan), 0.5% (w/v) potassium dextran sulphate and 0.3 M sucrose in White's modified solution (White, 1965) with the pH adjusted to 5.8. After treatment with enzyme for 1 h at 30°C under stationary conditions, isolated protoplasts were filtered through 50 μm nylon mesh and washed three times with White's medium containing 0.3 M sucrose (washing solution) by centrifugation at 100 g for 5 min. In some experiments centrifugation was not employed.

Cell culture and drug treatments

Pollen tetrads extruded from anthers were cultured in White's medium supplemented with 0.05% (w/v) yeast extract and 0.3 M sucrose, at pH 5.8 (Ito and Stern, 1967).

Anticytoskeletal drugs were added from the start of culture. Cytochalasin B (Sigma Chemical Co., St Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 2 mg ml⁻¹. The stock solution was added to the culture medium to give final concentrations of 1, 10 and 100 μM. Addition of DMSO alone to the medium (below 5% (v/v)) had no appreciable effect on the cultured cells. Colchicine (Wako Pure Chemical Industries, Ltd, Osaka, Japan) dissolved in distilled water was used at concentrations of 0.1, 1 and 10 mg ml⁻¹. All cultures were maintained at 25°C for 2 days.

DNA staining

The cells extruded from anthers, the isolated protoplasts and the cultured tetrads were fixed in 1% (v/v) glutaraldehyde in washing solution for more than 30 min, and were then stained with 1 μg ml⁻¹ 4′,6-diamidino-2-phenylindole (DAPI, Sigma) dissolved in NS buffer containing 0.3 M sucrose (washing solution) by centrifugation for 10 min. The stock solution was added to the culture medium to give final concentrations of 1, 10 and 100 μg ml⁻¹. Addition of DMSO alone to the medium (below 5% (v/v)) had no appreciable effect on the cultured cells. Colchicine (Wako Pure Chemical Industries, Ltd, Osaka, Japan) dissolved in distilled water was used at concentrations of 0.1, 1 and 10 mg ml⁻¹. All cultures were maintained at 25°C for 2 days.

Microtubule staining

The intact and cultured cells were fixed in 3% (w/v) paraformaldehyde in microtubule-stabilizing buffer containing 50 mM Pipes (pH 7.3), 10 mM EGTA, 5 mM MgCl₂ and 0.3 M sucrose for 30 min. They were then allowed to settle and washed twice in buffer without fixative. Samples were then affixed to coverslips coated with 0.1% (w/v) poly-l-lysine (M, 70,000–150,000, Sigma) and were often squashed beneath another non-coated coverslip. Freshly isolated protoplasts were affixed directly to the poly-l-lysine coated coverslips and fixed in methanol (~20°C). After washing in ice-cold buffered saline (BBS) containing 162 mM H₂BO₃, 35 mM NaOH and 144 mM NaCl (pH 8.1) supplemented with 0.05% (w/v) Triton X-100, the coverslips were exposed to mouse monoclonal anti-chick brain α-tubulin antibody (Amersham, Buckinghamshire, England) at a final concentration of 1.50% (v/v) for 1 h at 37°C in a moist chamber. Following further washing in BBS with 0.05% (w/v) Triton X-100, the samples were labeled with a 1:25 (v/v) dilution of fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Amersham, Buckinghamshire, England) for 1 h at 37°C in a moist chamber. After washing in PBS, samples were stained with 1 μg ml⁻¹ DAPI for 15 min, and finally washed in phosphate-buffered saline (PBS), and mounted in glycerol (10% (v/v) glycerin in PBS).

Actin staining

All samples were suspended in the washing solution and were directly stained with 0.03 μM rhodamine–phalloidin (Molecular Probes, Eugene, OR, USA) dissolved in PBS and 1 μg ml⁻¹ DAPI on a glass slide for more than 1 h.

Fluorescence microscopy

Stained cells were observed under an Olympus BHS-RPK epifluorescence microscope equipped with phase contrast. Preparations were viewed under ultraviolet irradiation (a UV-1 excitation filter and an L 435 barrier filter) for DAPI, blue-light
irradiation (BP 490 and EY 455 excitation filters and an O 515 barrier filter) for FITC, and green-light irradiation (BP 545 and EO 530 excitation filters and an O 590 barrier filter) for rhodamine. Fluorescence micrographs were usually taken on Fuji Neopan F film (ASA 32) for DAPI and Kodak Tri-X Pan film (ASA 400) for FITC and rhodamine.

Results

Behavior of plastid nucleoids during meiotic division

Fig. 1 shows the presence and distribution pattern of nuclear and organellar DNA in a microsporocyte at each stage of meiotic division, as visualized by fluorescence microscopy after staining with DAPI. Since the cell wall of the microsporocytes had practically no fluorescence, the appearance of cell nuclei and the numbers of cells as visualized by light microscopy were used to identify each stage. The fluorescence from plastid nucleoids could not be completely distinguished from that of mitochondrial nucleoids. However, it is known that plastid nucleoids are larger and fluoresce more intensively than mitochondrial ones (Miyamura et al. 1987) and, in fact, the fluorescence from mitochondrial nucleoids was only detectable here in squash preparations at high magnification (Fig. 2). Because of the very faint fluorescence from the mitochondrial nucleoids, it was concluded that the distinct fluorescent dots observed in the cytoplasm in Fig. 1 indicated the presence of plastid nucleoids.

The size and distribution of plastid nucleoids changed, in parallel with the behavior of the cell nuclei during meiosis. Plastid nucleoids were present in premeiotic cells (Fig. 1A), but their number seemed to increase after the onset of meiosis. In leptotene and zygote stage, many smaller-sized plastid nucleoids were observed throughout the cytoplasm (Fig. 1B,C). During pachytene and diplotene stages the size of the plastid nucleoids gradually increased (Fig. 1D,E), remaining at a maximum until diakinesis (Fig. 1F). At metaphase I, anaphase I and prophase II the larger nucleoids were distributed at random in the cytoplasm of the nearly spherical cells (Fig. 1G,H). From anaphase I to telophase I the plastid nucleoids migrated between two daughter nuclei. At telophase I, when the two daughter nuclei were polarized at the ends of each protoplast, the radiating microtubules were very striking (Fig. 3L). They showed that the plastid nucleoids, in addition to cortical, cytoplasmic and spindle microtubules (Fig. 3A-C), peculiar microtubules radiating out from the entire nucleus were observed at telophase I, interphase II, telophase II and the pollen tetrad stage (Fig. 3D,F,H and J). At telophase I and II when the two daughter nuclei were polarized at the ends of each protoplast, the radiating microtubules were predominantly arrayed towards the cell plate where phragmoplasts were formed (Fig. 3D,H). Double staining for microtubules and DNA suggested that the radiating microtubules played a role in the specific plastid arrangements (Fig. 3E,I). At interphase II and pollen tetrad stage the presence of radiating microtubules also seemed to result in the exclusion of the plastids from around the cell nuclei (Fig. 3F,G,J and K).

In squashed microspores without enzyme treatment the radiating microtubules were very striking (Fig. 3L). They seemed to radiate out uniformly from the nuclear surface to the plasma membrane and to be less dense at the two poles that were furthest away from the nucleus. Double staining showed that the plastid nucleoids were concentrated in the pole regions where the radiating microtubules were less dense (Fig. 3L,M). Soon after liberation from tetrads, visualization of the microtubule cytoskeleton was difficult because of the presence of the thick pollen exine.

Actin organization during microsporogenesis

Throughout meiotic division actin filaments were observed in the cytoplasm of intact cells not treated with fixative. This was consistent with previously reported results in *Lilium* (Sheldon and Dawes, 1988). They usually formed a network, regardless of the behavior of the chromosomes (Fig. 4A-C). At telophase I, interphase II,
Fig. 1. Behavior of plastid nucleoids during male meiosis in *Lilium longiflorum*. Fluorescent dots in the cytoplasm show the presence of plastid nucleoids, visualized with DAPI staining. (A) Premesiotic interphase. (B) Leptotene. (C) Zygotene. (D) Pachytene. (E) Diplotene. (F) Diakinesis. (G) Metaphase I. (H) Anaphase I. (I) Telophase I. (J) Interphase II. (K) Prophase II. (L) Metaphase II. (M) Anaphase II. (N) Telophase II. (O) Early-tetrad stage. Arrows in I and N show the cell plate. Non-random distribution of the plastid nucleoids is evident in I, J, N and O. Bar, 20 μm.
Fig. 2. Cell polarity of plastid nucleoids in young microspores of Lilium longiflorum. All cells are stained with DAPI, and viewed by fluorescence microscopy except in C. (A) Mid-tetrad stage. (B) Young microspores with thin exine at late tetrad stage. (C, D) A young microspore under phase-contrast (C) and fluorescence (D) optics. In B and D plastid nucleoids (large arrows) are polarized at the two poles, whereas mitochondrial nucleoids (small arrows) are scattered throughout the cytoplasm. (E–G) Protoplasts isolated from pollen tetrads. The plastid nucleoids are absent from the periphery of the cell nuclei. Bars, 20 μm.

telophase II and pollen tetrad stage when distinct plastid polarity was recognized, the actin filaments were randomly distributed in the cytoplasm (Fig. 4D,E). The random distribution of actin filaments was also preserved in isolated protoplasts (Fig. 4F), although the plastid showed polarized distribution. This indicates that the actin cytoskeleton was not involved in the organization and distribution of the plastid nucleoids.

Effects of anticytoskeletal drugs on the plastid arrangements (Fig. 5)
When pollen tetrads were cultured in a nutrient medium for 2 days, plastid polarity was retained in nearly all the microspores, although the cells were somewhat enlarged (Fig. 5A). Addition of 1 or 10 mg ml⁻¹ colchicine to the nutrient medium disrupted plastid polarity, resulting in a random distribution of plastids (Fig. 5B). Double staining
Fig. 3. Organization of microtubule cytoskeleton during microsporogenesis in *Lilium longiflorum*. All cells were doubly stained with anti-tubulin antibody and DAPI. (A–D, F, H, J, L) Immunofluorescence; (E, G, I, K, M) DAPI staining. Images for DAPI are eliminated in A–C. (A–K) Protoplasts isolated at prophase I (A), metaphase I (B), metaphase II (C), telophase I (D, E), interphase II (F, G), telophase II (H, I) and pollen tetrad stage (J, K). (L, M) A young microspore. Microtubules radiating from the cell nuclei are seen in D, F, H, J and L. In D there are distinct radiating microtubules independent of phragmoplast microtubules. In L some of the radiating microtubules reach the plasma membrane. Thick arrows show the presence of plastid nucleoids, where the radiating microtubules from the nuclei are less dense. Bars, 20 μm.
Fig. 4. Organization of the actin cytoskeleton during microsporogenesis in *Lilium longiflorum*. All cells were doubly stained with rhodamine-phalloidin and DAPI. (A–D, F) Rhodamine-phalloidin staining; (E) DAPI staining. (A) Metaphase I. (B) Metaphase II. (C) Pollen tetrad. (D, E) Interphase II. (F) An isolated protoplast from pollen tetrads. Actin filaments form an extensive cytoplasmic network irrespective of the behavior of the chromosomes and the plastid polarity. Bar, 20 μm.

Discussion

**Visualization of plastid nucleoids by DAPI staining**

In this study, sequential changes in the distribution of plastid and mitochondrial nucleoids during male meiosis were followed using fluorescence microscopy after staining with DAPI. The behavior of these organelles during male meiosis has mainly been documented ultrastructurally (Maruyama, 1968; Dickinson and Heslop-Harrison, 1970; Bird *et al.* 1983). Observations of ultrathin sections are not able to show the polarity and distribution of the total organelar population. Observation of these organelles in the light microscope is also limited. The use of DAPI staining enables plastids and mitochondria to be clearly visualized by the fluorescence of the DNA in their nucleoids.

It is difficult to distinguish between plastid and mitochondrial nucleoids by differences in the intensity of fluorescence, although it has been reported that plastid DNA fluoresces more strongly (Coleman, 1978, 1979; James and Jope, 1978; Kuroiwa *et al.* 1981). Miyamura *et al.* (1987) also reported that in *Triticum aestivum* the plastid nucleoids could be distinguished from mitochondrial ones on the basis of differences in size using phase-contrast optics, and that during male meiosis the plastid nucleoids fluoresced more intensely than mitochondrial nucleoids. Also, enlargement of the larger dots during prophase I seemed to coincide with the fact that active DNA synthesis took place in plastids at the pachytene stage (Smyth, 1982). Further, localization of plastids at the two poles in young microspores of *Lilium* has been demonstrated by electron microscopy (Dickinson, 1981). This was also shown in the lily studied here (data not...
Fig. 5. Effects of anticytoskeletal drugs on the distribution of plastids in cultured pollen tetrads of *Lilium longiflorum*. Pollen tetrads were cultured for 2 days in drug-free medium (A, C, D), 10 mg ml\(^{-1}\) colchicine-containing medium (B, E, F) and 100 μg ml\(^{-1}\) cytochalasin B-containing medium (G–J). All cells were doubly stained with DAPI and anti-tubulin antibody or rhodamine-phalloidin. (A, B, D, F, H, J) DAPI staining; (C, E, I) immunofluorescence; (G) rhodamine-phalloidin staining. In the control culture, plastid polarity due to the presence of radiating microtubules was evident (A, C, D). Colchicine disrupted the plastid polarity by depolymerizing the radiating microtubules (B, E, F). In the colchicine-treated cell the microtubules are recognized only near the nuclear envelope. Disappearance of actin filaments caused by cytochalasin B had no effect on the plastid polarity (G, H), and the microtubules remained in the presence of cytochalasin B (I, J). Bars, 20 μm.

shown). Therefore it was concluded that the larger, more intensively fluorescing dots were plastid nucleoids.

**Appearance of plastid polarity during male meiosis**

Although slightly squashed preparations were used to examine plastid nucleoids, a non-random pattern of distribution was distinct at specific stages, and this was not affected by cell wall digestion and centrifugation procedures. At telophase most plastid nucleoids aggregated at the equatorial region of the cell. Such a migration of plastids to the equator at telophase has been reported in other plant species (Wolniak, 1976; Bednara et al. 1986; Tanaka, 2003).
Rodkiewicz et al. 1986), but in these mitochondria migrate together with the plastids. In this study polarity in the distribution of the plastids was recognized only at telophase I, interphase II, telophase II and tetrad stage (Figs 1, 2).

The finding that from telophase I the appearance of plastid polarity was restricted to telophase and interphase suggested that the phenomenon was not related to the formation of spindles and chromosomes, but to the presence of the nuclear envelope. Further, it is assumed that the position of the nucleus in the cell is an important factor in the distribution of plastids.

Function of microtubules in the distribution of plastids

The dynamics of microtubule organization during male meiosis has already been demonstrated, using immunofluorescence, in several angiosperms (Van Lammeren et al. 1985; Sheldon and Dickinson, 1986; Hogan, 1987; Traas et al. 1989) and mosses (Brown and Lemmon, 1987a,b; Busby and Gunning, 1988). These studies showed that microtubules functioned in nuclear division and cytokinesis during male meiosis through the formation of spindles and phragmoplasts. This was also found to be the case in the present study.

Microtubules that radiate out from the cell nuclei have been observed during microsporogenesis in several angiosperms, but no clear function has been demonstrated. This radial system of microtubules was first observed in protoplasts isolated from young microsperms of Lilium by Dickinson and Sheldon (1994). They suggested that these radiating microtubules may function in cytoplasmic transport. Subsequently it was suggested that the radiating microtubules were involved in nuclear positioning (Van Lammeren et al. 1985; Hogan, 1987) or in the establishment of the future division plane (Traas et al. 1989).

In this study, it was clear that the radiating microtubules observed at telophase I, interphase II, telophase II and pollen tetrad stage functioned in maintaining the specific distribution of plastids. Although double staining with anti-tubulin antibody and DAPI reduced the resolution of the plastid nucleoids, there was obviously a close relationship between the radiating microtubule arrays and the distribution of the plastid nucleoids. The correlation between the radiating microtubules and plastid arrangement was clearest in young microsperm from the mid-tetrad stage (Fig. 3L,M). The microtubules seemed to radiate out uniformly from the nuclear surface to the plasma membrane and to be less dense at the two poles, which would result in the peculiar arrangement of plastids in the ellipsoidal cell, in which they were situated at the two poles, furthest away from the nucleus. The role of the radiating microtubules in the arrangement of the plastids was ascertained by colchicine treatment of pollen tetrads, which lacked spindle and phragmoplast microtubules. Colchicine treatment resulted in the disappearance of the radiating microtubules, and the disruption of the plastid arrangement (Fig. 5B,E and F). It was therefore concluded that the presence of radiating microtubules resulted in exclusion of plastids from around the cell nuclei. However, it was unclear how the radiating microtubules excluded the plastids. The plastids might be transported to the distal ends of the microtubules.

In telophase cells, the function of radiating microtubules was complicated because of the presence of phragmoplast microtubules. The plastids were aggregated in the equatorial regions of the cells, coincident with the phragmoplast microtubules. The radiating microtubules from the cell nuclei are predominantly arrayed towards the equators of the cells where phragmoplasts were formed, and were longer between the two nuclei (Fig. 3D,H). However, there were distinct radiating microtubules, independent of the phragmoplast microtubules. Such microtubule arrangement at telophase is also seen in other microsporocytes (Sheldon and Dickinson, 1986; Hogan, 1987; Traas et al. 1989). Euteneuer and McIntosh (1989) showed that the phragmoplast in Haemanthus endosperm was formed at the junction where the distal ends of the microtubules from two daughter nuclei were overlapping. Although the phragmoplast microtubules had originated from the daughter nuclei, they were shortened so as to become concentrated at the equator at late telophase in mitotic cells (De Mey et al. 1982). Unlike the somatic cell phragmoplast, the radiating microtubules in meiotic cells remained stable after completion of cell plate formation. Further, the radiating microtubules appeared between two daughter nuclei at telophase in tomato when phragmoplast formation did not occur (Hogan, 1987). Therefore, it was assumed that the plastids were scattered in the equatorial region, the furthest distance from the daughter nuclei, due to the presence of the radiating microtubules. The relationship between the radiating microtubules and the phragmoplast microtubules was uncertain. Because the migration of plastids into the equator seemed to occur before phragmoplast formation, the radiating microtubules arrayed towards the equator might form the phragmoplast after transporting the plastids to the equator.

The radiating microtubules were detected in telophase and interphase cells. This suggests that the radiating microtubules originated from the nuclear envelope and that the nuclear envelope functioned as the microtubule organizing center (MTOC). De Mey et al. (1982) and Dickinson and Sheldon (1994) have suggested that microtubule organizing activity is associated with the nuclear envelope. It has also been suggested that microtubules in higher plant cells are organized from zones or areas of the nucleus (Clayton et al. 1985). In the present study it was clear that the radiating microtubules for the most part originated from the entire nucleus (Fig. 3L). The extensive organization of the radiating microtubules would also account for the change in plastid polarity caused by migration of the cell nuclei from the periphery to the center during interphase II and pollen tetrad stage.

Such an arrangement of organelles based on microtubules is well established in animal cells (Stebbins, 1990), and in lower plant cells an interaction between plastids and microtubules has also been shown (Menzel, 1985; Busby and Gunning, 1988). To the author's knowledge this study is the first demonstration of a microtubule-based plastid arrangement in higher plant cells.

Independence of actin filaments in the distribution of plastids

It has been suggested that actin filaments might function in the movement and distribution of organelles (Menzel and Elsner-Menzel, 1989; Van Lommelen et al. 1990). The dynamics of actin organization during male meiosis was recently revealed by rhodamine-phalloidin staining (Sheldon and Hawes, 1988; Traas et al. 1989; Van Lommelen et al. 1990). I therefore examined the organization of the actin cytoskeleton throughout microsporogenesis. My results were not always consistent with...
previous reports in terms of the association of actin filaments with the spindle and the phragmoplast. However, the organization of actin in dyads and pollen tetrads was found to be the same as previously reported, in that the actin filaments were oriented at random throughout the cytoplasm. In the dyads and pollen tetrads though, polarity of plastid distribution was clear. Further, disappearance of actin filaments caused by cytochalasin B had no effect on the plastid polarity in the cultured pollen tetrads. Therefore, it was concluded that actin filaments do not function in the distribution of plastids.

**Significance of the radiating microtubules in male gamete formation**

The microspores derived from male meiosis soon divide to give generative and vegetative cells. The composition of the cytoplasm of the generative cell, which is the progenitor of two male gametes in angiosperm plants, is very different from that of the vegetative cell. Ultrastructural studies have shown that the generative cells of several species, including the lily studied here, lack plastids (Heslop-Harrison, 1968; Sanger and Jackson, 1971; Clauss and Grun, 1977; Rodriguez-Garcia and Garcia, 1978; Nakamura and Miki-Hirosige, 1985; Schroeder, 1986; Tanaka et al. 1989). These studies suggested that plastids were maternally inherited in these species. Although there may be a number of ways by which plastids are excluded from the male gametes (Sears, 1980; Hagemann and Schroeder, 1986), it is clear that it is the first mitosis that results in all or almost all of the plastids being absent from the generative cell. The first mitosis is a polar one, in which the microspore nucleus migrates to one end of the cell. If the radial system of microtubules were inherited through the first mitosis, and accounted for the exclusion of plastids from around the nucleus, it would seem likely that the generative cell formed at one end would not contain plastids. The arrangement of plastids and organization of the microtubule cytoskeleton during the first mitosis in microspores are therefore now being investigated, using protoplasts isolated from pollen grains (Tanaka et al. 1987).

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