Actin Dynamics during Pollen Ontogeny of *Tradescantia paludosa*

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Actin dynamics were studied by rhodamine-phalloidin method during the pollen ontogeny of *Tradescantia paludosa*. During the interphase and prophase of the microspore division, some filamentous (F-) and amorphous actin existed in the cortical cytoplasm or associated around the nucleus. No F-actin was observed in the metaphase cell. During telophase, F-actin was constructed into the phragmoplast which developed curving toward the generative pole and disappeared with phragmoplast disorganization. Following microspore division, F-actin and actin bundles appeared on both transverse sides of the vegetative cell nucleus and developed into a complex network. This actin was reformed into fine filaments and filled in the vegetative cell within the mature pollen grain. The fine filaments were migrated into the pollen tubes and reconstructed into one of several different forms, a thick bundle, fine filament or intermediate form, depending upon tube growth. In some pollen tubes, F-actin formed helical or circular bands in a mid- or tip region. No F-actin was observed in both generative and sperm cells. In microspores and pollen grains treated with cytochalasin B, which is an inhibitor of actin polymerization, the growth of the pollen tube was completely inhibited, but the two nuclear migrations, curving development of phragmoplast in the microspore cell and generative cell elongation were not affected. To conclude, then, the major function of F-actin developed in the pollen grain was to contribute to tube growth.

The actin behaviors in the present study are compared with microtubules in our previous paper and the distinctly different dynamics of the two will be discussed.

Key words: Actin, Cytochalasin B, Cytoskeleton, Pollen ontogeny, *Tradescantia paludosa*.

Introduction

Pollen, a male gametophyte of flowering plants, is a suitable material for studies on cell division, differentiation and growth. During the pollen ontogeny of angiospermous plants, some peculiar phenomena occur in succession : nuclear migration before microspore division, unequal division of the microspore, chromatin and cell differentiations between two daughter cells, *i. e.*, generative and vegetative cells, generative and vegetative cell elongations (the latter called pollen germination) and generative cell division[1]. The nuclear migration is the major prerequisite for the subsequent unequal cell division, and unequal division is essential for chromatin and cell differentiations between the two daughter cells[2]. The present authors previously investigated microtubule (MT)

dynamics during pollen development by immunofluorescence methods and reported that the nuclear migration was mediated by MT cytoskeleton. Many more MTs were distributed in the generative than the vegetative cell, and MTs played an important role in generative cell elongation[1]. In the pollen tube, some peculiarly formed spindles were organized in the generative cell divisions of some species[3-5].

Recently, actin cytoskeleton was reported as present in most plant cells, including pollen grain cells and pollen tubes. Van Lammeren *et al*[6] reported that F-actin formed parallel arrays in the peripheral cytoplasm of the microspores and pollen grains in *Gasteria verrucosa*. In dehydrated mature pollen grains of some species, actin was observed in fusiform, spicular troidal or circular form, which became filamentous during hydration[7-11]. F-actin was shown to exist in the pollen tube and participate in cytoplasmic streaming and tube growth[12-14]. It was reported that an actomyosin system related to cytoplasmic streaming, or the motility of vegetative nucleus, generative cell and other cell organelles, was present in angiospermous pollen[11,15-17]. The co-localization of MTs and F-actin was also reported in the pollen tubes of some species and many other plant cells, suggesting a functional association between them[12,14,18-20]. However, no definitive evidence of their functional association was offered. Actin behavior during pollen grain development is also little known, because the thick pollen wall poses a barrier to the fluorescence method. In the present study, actin dynamics throughout the entire process of the pollen ontogeny of *Tradescantia paludosa*, of which the pollen wall is comparatively thinner, was investigated using the rhodamine-phalloidin method, and the results obtained compared with the MT dynamics previously reported by the present authors in order to clarify their distributional and functional relationships.

Materials and Methods

Pollen grains of *Tradescantia paludosa* were used in the present study. Stalks with anthers growing at some adequate stages were placed in 10 μg/ml cytochalasin B solution, which serves as an inhibitor of actin polymerization, for 2 days. Mature pollen grains were cultured in the medium described by Kwack and Kim[21]. Pollen grains and pollen tubes were fixed for 1 hr with 4% formaldehyde in 50 mM potassium phosphate buffer solution with 5 mM EGTA, pH 6.9. The fixed pollen tubes were air-dried on the cover glass for more than 1 hr. Materials were rinsed in the buffer solution for 1 hr. The pollen grains were then exposed in the intine-digestive enzyme solution (1% cellulase, 1% macerozyme and 0.1M sucrose) for 1 hr, and the pollen tubes for 15 min. After being rinsed again in the buffer solution for 30 min, the pollen grains were smeared on a 0.1% poly-L-lysin coated cover glass and air-dried for more than 1 hr. The pollen grains and pollen tubes were treated with 0.5% Triton X-100 in phosphate buffered saline (PBS, Sigma) for 1 hr and rinsed in PBS for 10 min. Actin was then stained with rhodamine-phalloidin (Transformation Research Inc.) diluted 1 : 10 with PBS, for 45 min at room temperature. After being rinsed again in PBS for 30 min, nuclei in pollen grains and pollen tubes were stained with 1 μg/ml 4'6'-diamidino-2 phenylindole (DAPI) in PBS for 5 min to examine their exact mitotic figures. Fluorescence of the samples was observed with a Nikon Microphoto-FX microscope and photographed on Kodak Tri-X pan film.

Results

The microspores and pollen grains of *T. paludosa* were ellipsoidal or semicircular. The interphase nucleus in the microspore cells migrated twice before microspore division; first, from the center to one side, and second, from the side to the center near the inner wall of the tetrad. A vacuole developed before and after the first nuclear migration and occupied a large part of the cell. Just after the second nuclear migration, the microspore divided along its short axis. The microspore division, which was distinctly unequal, produced a small generative cell near the inner wall and a large vegetative cell on the opposite side. During pollen grain maturation, the generative cell elongated extremely in the vegetative cell cytoplasm.

The appearances of actin in the interphase microspore are shown in Fig. 1. In the microspore just after release from the tetrad, a few amorphous fluorescent images were observed in the cortical region (Fig. 1A). After the first nuclear migration, actin showed a filamentous or bundle configuration in the peripheral cytoplasm around the vacuole (Fig. 1B). The bundles of F-actin slightly increased before the second nuclear migration and associated with nucleus, facing the vacuole (Fig. 1C). After the second nuclear migration, the actin bundles began to disorganize into fine filamentous or amorphous actin, but its distribution pattern did not change notably (Fig. 1D). "Amorphous" actin was included in no distinct filamentous configuration under the fluorescence microscope. Actin tended to localize toward one side of the nucleus during the prophase of microspore division (Fig. 1E). In the metaphase cell, F-actin was completely disorganized into an amorphous actin and diffused throughout the cytoplasm (Fig. 1F). No F-actin was observed in the metaphase spindle. At late anaphase, organized actin began to reappear and accumulated in the cytoplasm between the two daughter chromosomes (Fig. 1G). F-actin was constructed into the phragmoplast and around the vegetative nucleus at telophase (Fig. 2A). The phragmoplast developed curving toward the generative nucleus (Fig. 2B). After microspore division, actin disappeared from the phragmoplast, and coincidentally, F-actin began to be organized on both transverse sides of the nucleus in the vegetative cell (Fig. 2C). F-actin formed thick bundles. The number of these bundles increased abundantly, orientating in random directions, and developed into a complex network (Fig. 2D-F, 3A). No actin was observed, however, in generative cells. During pollen grain maturation, the actin bundles in the vegetative cell were successively changed into fine filaments (Fig. 3B). At the matured stage, very fine F-actin filled in the vegetative cell cytoplasm (Fig. 3C). The extremely elongated generative cell contained almost no actin.

The mature pollen grains were incubated in the culture medium at 25℃. Almost all pollen grains germinated within 15 min. Abundant fine F-actin was migrated from the pollen grain into the germinated pollen tube. This actin began to be reorganized into some different configurations in accordance with pollen tube growth. In many pollen tubes placed in the culture for 45 min, some F-actin bundles appeared among fine F-actin. In 1.5 hr culture, the F-actin bundles increased in number within a pollen tube (Fig. 4A), and after more than 2 hr, they were more developed, whereas fine F-actin decreased (Fig. 4B). In the apical regions of elongating pollen tubes, almost no, or only a small amount of, amorphous actin was present. In the sub-apical regions, from the tip to the proximal direction, only amorphous, small globular-shaped and fragmentary actin was observed (Fig. 4C). Almost all the F-actin and bundles arrayed along the long axis of the pollen tube. In a few pollen tubes the thick bundle showed a partially helical

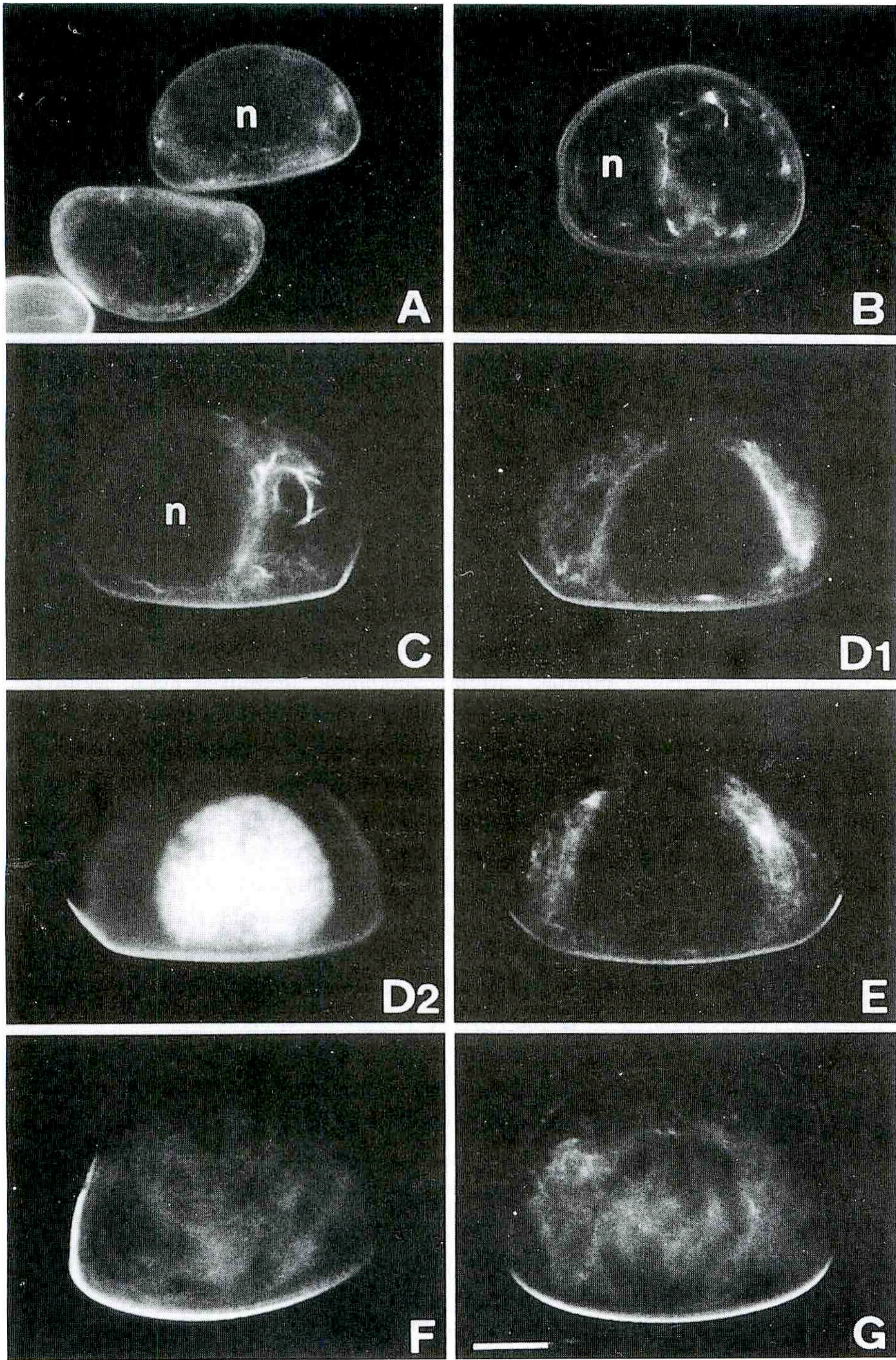

Fig. 1

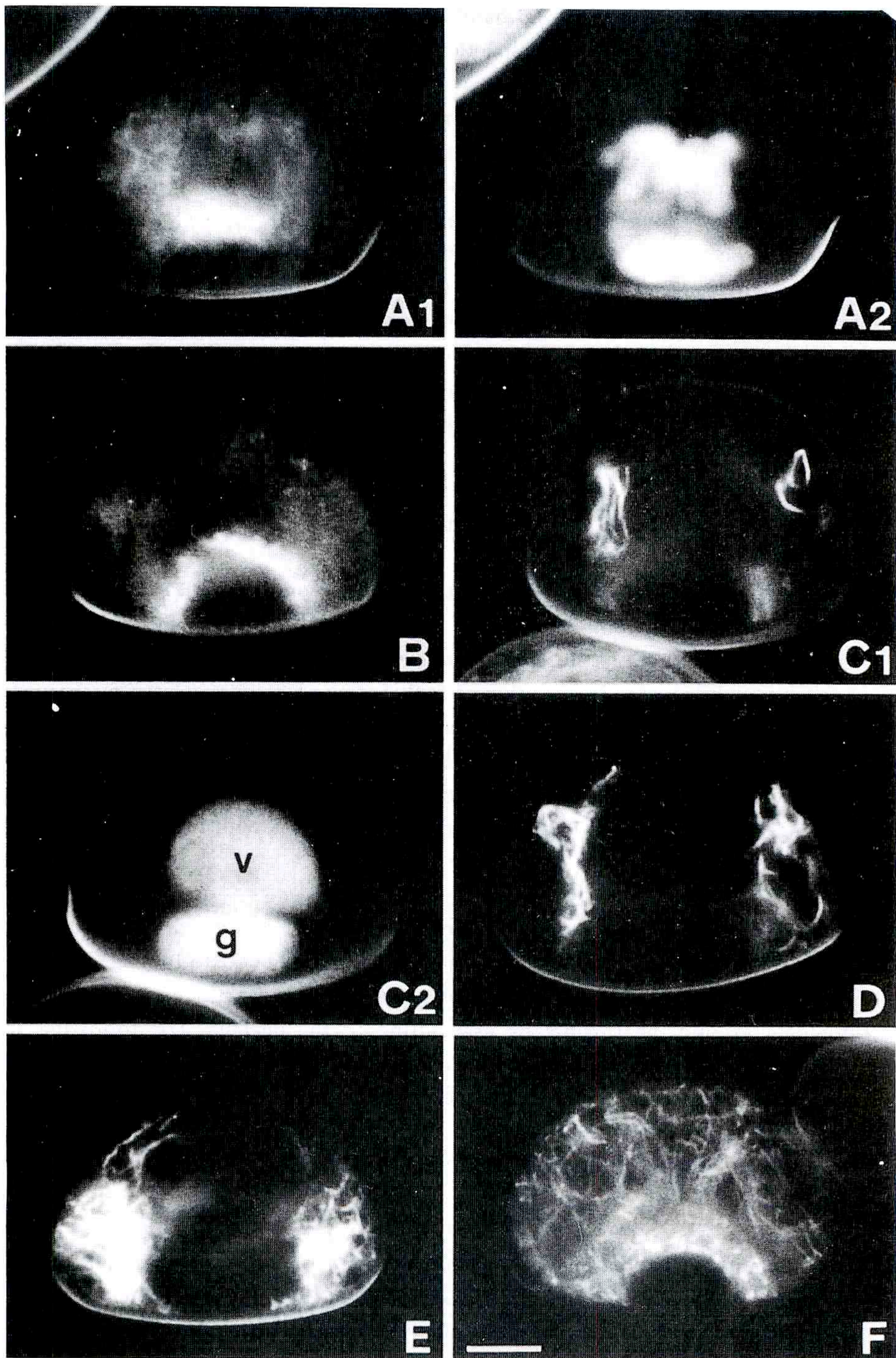

Fig. 2

arrangement (Fig. 4D), and near the growing tip region, circular bands of cortical F-actin were formed across the pollen tube long axis (Fig. 4E). In many cases, the tip regions bearing those bands swelled abnormally. The circular bands seemed to be first organized at the apical part and maintained in the mid-region following the continuous elongation of the pollen tube. No actin was detected in either generative or sperm cells in the pollen tubes.

The developing microspores were treated with 10 μg/ml cytochalasin B solution for 2 days. This was done in order to examine the relationship between actin dynamics and the two nuclear migrations in the interphase microspore cell and subsequent aspects. In many treated pollen grains, F-actin was disorganized into an amorphous form. The nucleus, however, normally displaced twice, and proceeded to the mitosis in the native site near the inner wall (Fig. 5A-C). The phragmoplast developed curving toward the generative pole, although actin fluorescence in the phragmoplast became indistinct (Fig. 5D). The unequal cell division and the subsequent generative cell elongation were performed by means of normal manners (Fig. 5E, F). In the mature pollen grains cultured for more than 1 hr in the medium with 10 μg/ml cytochalasin B, F-actin became fragmentary, and the pollen did not germinate at all.

Discussion

In the present study, actin dynamics throughout the pollen ontogeny of *T. paludosa* was studied and compared with the MT dynamics previously reported by the present authors[1]. In the present species, the two migrations of the nucleus occurred regularly in the interphase microspore cell. The second migration was major prerequisite for the unequal division of the microspore and the generative cell elongation. Some F-actin appeared in the cell periphery or around the nucleus before and after the two nuclear migrations. However, no significant change in its distribution corresponding to the nuclear migrations occurred, nor migrations were inhibited by cytochalasin B. From these results, it was concluded that actin did not associate with the two nuclear migrations

Fig. 1. **Actin distribution during the microspore division of *T. paludosa*.** A-D, interphase microspores. A, just after release from the tetrad. B and C, following the first nuclear migration. A small amount of amorphous actin and F-actin existed in the peripheral cytoplasm around the vacuole or associating to the nucleus. D1, following the second nuclear migration. Some amorphous and fine F-actin localized on one side of the nucleus. D2, DAPI fluorescence figure of the cell shown in D1. E, prophase in the microspore division. F, metaphase. G, anaphase. Amorphous actin and F-actin just beginning to accumulate in the cytoplasm between the two daughter chromosomes. n, microspore nucleus. Bar, 10 μm.

Fig. 2. **Actin distribution during the microspore division.** A1, early telophase ; B, mid-telophase. Actin was constructed in the phragmoplast which developed curving toward the generative nucleus. C1, late telophase. Distinct F-actin began to be organized on both sides of the vegetative nucleus. D-F, bi-cellular stage. F-actin and actin bundles increased and formed a complex network. A2 and C2, DAPI fluorescence of the same cells shown in A1 and C1, respectively. v, vegetative cell nucleus; g, generative cell nucleus. Bar, 10 μm.

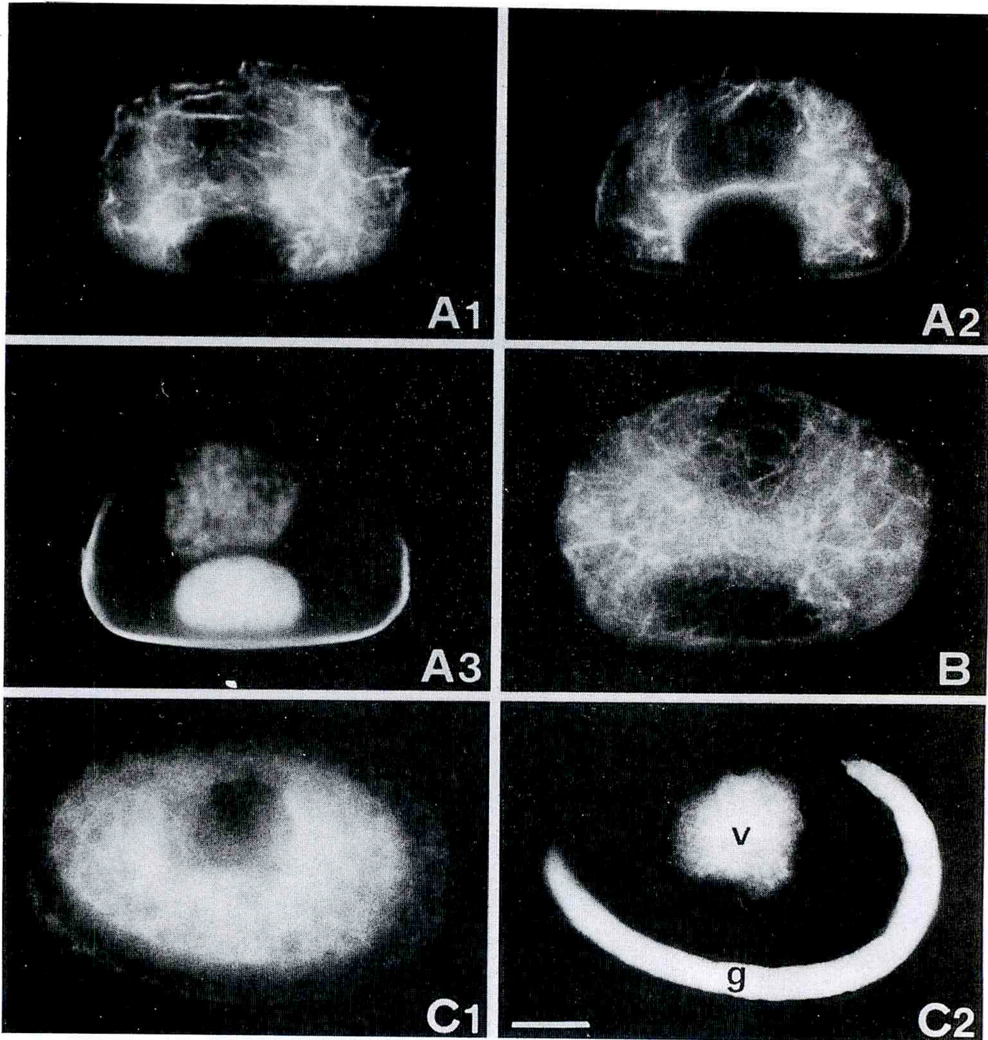

Fig. 3. Actin distribution during pollen grain maturation. A1-A3, the same pollen grain. A1 was the figure focused on the cell surface and A2 the figure focused on the nuclei. A3 was the DAPI stained figure. B, premature and C1, mature pollen grains. F-actin and actin bundles took on a very fine filamentous form. C2, DAPI fluorescence figure of the cell shown in C1. Bar, 10 μm.

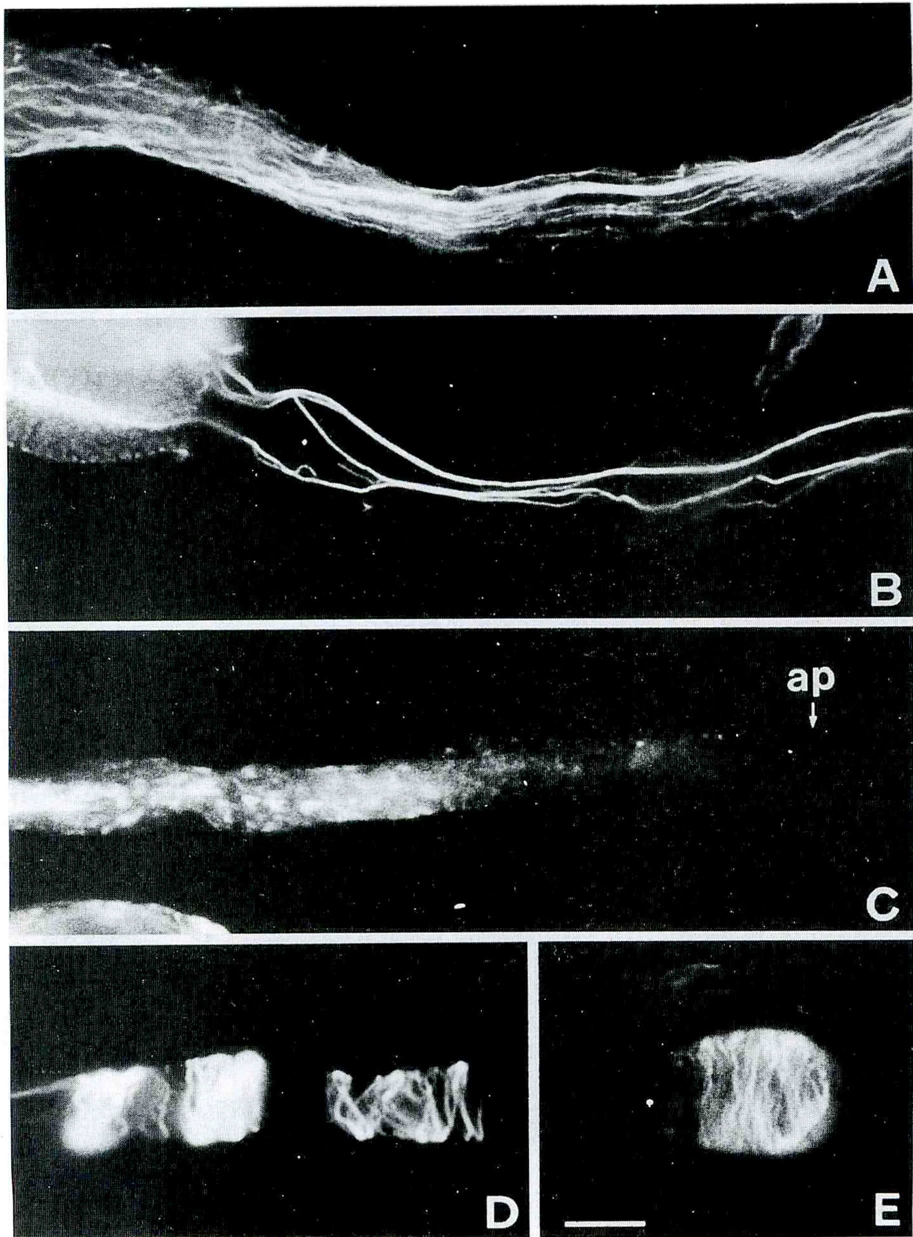

Fig. 4. Actin arrangements in the pollen tubes. A, F-actin and F-actin bundles in the pollen tube cultured for 1.5 hr. B, thick bundles of F-actin (2 hr). C, amorphous, small globular and fragmentary actin in the sub-apical region, and almost no any actin in the apical region (ap) (1.5 hr). D, helix of actin bundles and E, circular bands of cortical F-actin in the tip region (2 hr). Bar, 10 μm.

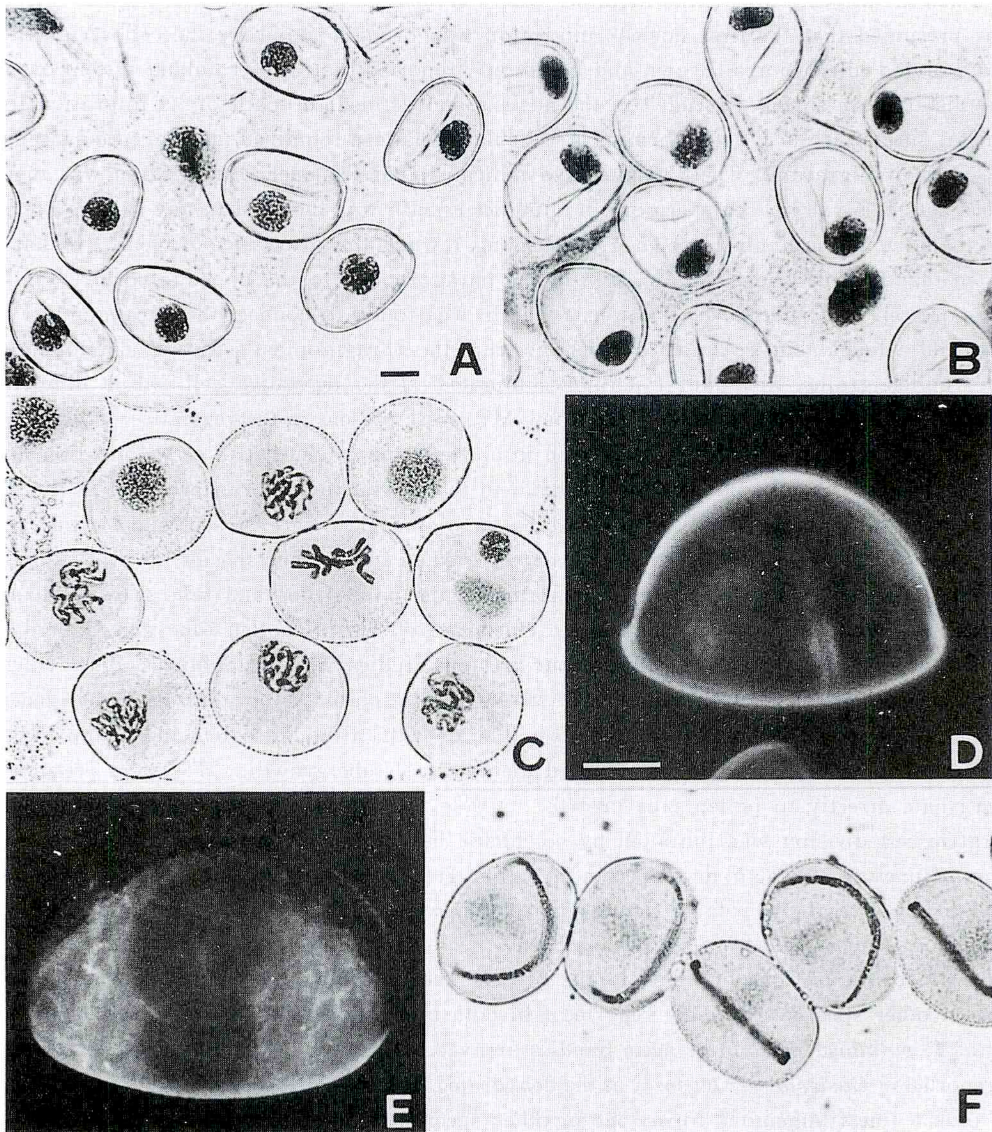

Fig. 5. Pollen grains treated with cytochalasin B. A, B, microspores before (A) and after (B) the first nuclear migration. C, microspores after the second nuclear migration and young pollen grains. The two nuclear migrations were not inhibited. D, mid-telophase of the microspore division. E, bi-cellular stage. The curving development of the phragmoplast and the unequal division took place normally, although F-actin had disorganized into amorphous form. Compare with Fig. 2B and Fig. 3A, respectively. F, mature pollen grains stained by aceto-carmine. Generative cells elongated normally. Bar, 10 μm.

in the microspore cell. The authors had previously reported that the second nuclear migration was mediated by MTs, which were organized and shifted in a manner corresponding to the second nuclear migration. Neither actin nor MTs were associated with the first nuclear migration. It was presumed that the first nuclear migration was brought about by the cell vacuolization. In moth orchid microspores, Brown and Lemmon[22] reported that a microtubule system organized at the distal spore surface extended to the nucleus and defined the path of its migration toward the distal surface while F-actin forming a reticulated pattern remained unchanged during preprophase nuclear migration. Their results also indicated that the nuclear migration was mediated by MTs but not by actin. Furthermore, no distinct F-actin was included in any mitotic apparatus other than the phragmoplast which grew curving toward the generative nucleus. It has been reported that F-actin bridged the margin of the phragmoplast to the opposite cortex[23,24]. In the microspore cell of *T. paludosa,* however, no actin was found to connect the phragmoplast to the cortex, and cytochalasin treatment did not interrupt the phragmoplast's curving development. In mature pollen grains, abundant F-actin accumulated in the vegetative cell, while almost none accumulated in the generative cell. Only a few MTs were present in the vegetative, but many were noted in the generative cell. MTs played an important role in generative cell elongation and the subsequent division in the pollen tube. F-actin in the vegetative cell was provided in the germinating pollen tube. In an elongated pollen tube, actin and MTs were present and showed similar arrangements. Their co-localization has been reported in some other species, as well, suggesting some co-functions[12,14,18,20]. Cytoplasmic streaming in the pollen tube and tube growth alike were inhibited by cytochalasin B[13,25-30], and some colchicine effects on pollen tube growth and shape have been reported[31,32]. But, according to our previous studies[33] and unpublished data, colchicine in a temperate concentration, about 5×10^{-4} M, enough to inhibit MT organization and generative cell division in the pollen tube, did not affect tube growth, although colchicine in too high concentration of more than 2.5×10^{-3} M reduced the extent of tube growth. MTs were presumed not to contribute directly to pollen tube growth. A role of MTs in a pollen tube remains unclear. Generative cell division was inhibited by colchicine but not by cytochalasin B. In conclusion, the major function of F-actin developed in the pollen grain was to contribute to pollen tube growth. The distribution and the role of actin cytoskeleton were distinctly different from those of MT cytoskeleton in many phases of pollen ontogeny.

The actin configurations observed in the present species were different in some aspects from studies of other species previously reported. In both microspore and vegetative cell in the pollen grain of *T. paludosa,* F-actin or actin bundles arrayed at random, although parallel arrangement was reported in *Gasteria verrucosa*[6]. In the dehydrated mature pollen grains of the present species, actin took a fine filamentous form, but in other species, fusiform or other peculiar forms have been reported[7-11]. The actin arrangement in the pollen tube of the present species generally resembled those of many other species previously reported. F-actin and actin bundles were arranged in parallel along the pollen tube for the most part, but in the tip region actin was amorphous or absent. Helical and circular arrangements of actin were also found in the pollen tubes of the present species at right angles to its long axis. A circular arrangement of cortical F-actin was reported around the subapical part of protonemal cells in the ferns *Adiantum capillus-veneris* and *Pteris vittata*[34] and the same arrangements of MTs and cellulose microfibrils (MFs) were also observed in *Adiantum*[35,36]. Murata and Wada[36] suggested the importance of MTs and MFs in the subapical region for the control of cell diameter. In the budgrowing subprotoplasts of *Nicotiana* pollen tubes, the base of the bud was often marked by a ring of cortical F-actin, and

most of the length of the outgrowth was occupied by circumferential F-actin which was oriented perpendicularly to the axis of elongation[37]. In the pollen tube, the circular arrangement of F-actin may be correlated to the abnormal swelling of the tip region in a culture condition. Definitive information on the role of circular F-actin remains unavailable.

References

(1) Terasaka, O. and T. Niitsu: Unequal cell division and chromatin differentiation in pollen grain cells. II. Microtubule dynamics associated with the unequal cell division. Bot. Mag. Tokyo **103**, 133-142 (1990).

(2) Terasaka, O. and T. Niitsu: Unequal cell division and chromatin differentiation in pollen grain cells. I. Centrifugal, cold and caffeine treatments. Bot. Mag. Tokyo **100**, 205-216 (1987).

(3) Terasaka, O. and T. Niitsu: Peculiar spindle configuration in the pollen tube revealed by the anti-tubulin immunofluorescence method. Bot. Mag. Tokyo **102**, 143-147 (1989).

(4) Terasaka, O. and T. Niitsu: Peculiar spindle configuration in the generative cell of *Tradescantia paludosa* by immunofluorescence method. Jap. J. Palynology **35**, 7-12 (1989).

(5) Palevitz, B. A. and M. Cresti: Cytoskeletal changes during generative cell division and sperm formation in *Tradescantia virginiana*. Protoplasma **150**, 54-71 (1989).

(6) Van Lammeren, A. A. M., J. Bednara and M. T. M. Willemse: Organization of the actin cytoskeleton during pollen development in *Gasteria verrucosa* (Mill.) H. Duval visualized with rhodamine-phalloidin. Planta **178**, 531-539 (1989).

(7) Cresti, M., P. K. Hepler, A. Tiezzi and F. Ciampolini: Fibrillar structures in *Nicotiana* pollen: Changes in ultrastructure during pollen activity and tube emission. Biotechnology and Ecology of Pollen. Mulcahy, D. L., Mulcahy, G. B., Ottaviono, E., eds. New York: Springer-Verlag. pp. 283-288 (1986).

(8) Heslop-Harrison, J., Y. Heslop-Harrison, M. Cresti, A. Tiezzi and F. Ciampolini: Actin during pollen germination. J. Cell Sci. **86**, 1-8 (1986).

(9) Pierson, E. S.: Rhodamine-phalloidin staining of F-actin in pollen after dimethylsulphoxide permeabilization: a comparison with the conventional formaldehyde preparation. Sex. Plant Reprod. **1**, 83-87 (1988).

(10) Tiwari, S. C. and V. S. Polito: Spatial and temporal organization of actin during hydration, activation, and germination of pollen in *Pyrus communis* L.: a population study. Protoplasma **147**, 5-15 (1988).

(11) Heslop-Harrison, J. and Y. Heslop-Harrison: Conformation and movement of the vegetative nucleus of the angiosperm pollen tube: association with the actin cytoskeleton. J. Cell Sci. **93**, 299-325 (1989).

(12) Lancelle S. A., M. Cresti and P. K. Hepler: Ultrastructure of the cytoskeleton in freeze-substituted pollen tubes of *Nicotiana alata*. Protoplasma **140**, 141-150 (1987).

(13) Perdue, T. D. and M. V. Parthasarathy: *In situ* localization of F-actin in pollen tubes. Europ. J. Cell Biol. **39**, 13-20 (1985).

(14) Pierson, E. S., J. Derksen and J. A. Traas: Organization of microfilaments and microtubules in pollen tubes grown *in vitro* or *in vivo* in various angiosperms. Europ. J. Cell Biol. **41**, 14-18 (1986).

(15) Tang, X., P. K. Hepler and S. P. Scordilis: Immunochemical and immunocytochemical identification of a myosin heavy chain polypeptide in *Nicotiana* pollen tubes. J. Cell Sci. **92**, 569-574 (1989).

(16) Heslop-Harrison, J. and Y. Heslop-Harrison: Myosin associated with the surfaces of organelles, vegetative nuclei and generative cells in angiosperm pollen grains and tubes. J. Cell Sci. **94**, 319-325 (1989).

(17) Kohno, T. and T. Shimmen: Accelerated sliding of pollen tube organelles along Characeae actin bundles regulated by Ca^{2+}. J. Cell Biol. **106**, 1539-1543 (1988).

(18) Pierson, E. S., H. M. P. Kengen and J. Derksen: Microtubules and actin filaments co-localize in pollen tubes of *Nicotiana tabacum* L. and *Lilium longiflorum* Thunb. Protoplasma **150**, 75-77 (1989).

(19) Tiezzi, A., A. Moscatelli, C. Milanesi, F. Ciampolini and M. Cresti: Taxol-induced structures derived from cytoskeletal elements of the *Nicotiana* pollen tube. J. Cell Sci. **88**, 657-661 (1987).

(20) Tiwari, S. C. and V. S. Polito: Organization of the cytoskeleton in pollen tubes of *Pyrus communis*: a study employing conventional and freeze-substitution electron microscopy, immunofluorescence, and rhodamine-phalloidin. Protoplasma **147**, 100-112 (1988).

(21) Kwack, B. H. and I. -H. Kim: An improved method for culturing *Tradescantia* pollen tubes for chromosomal analysis. Cytologia **32**, 1-5 (1967).

(22) Brown, R. C. and B. E. Lemmon: Pollen development in orchids. 3. A novel generative pole microtubule system predicts unequal pollen mitosis. J. Cell Sci. **99**, 273-281 (1991).

(23) Kakimoto, T. and H. Shibaoka: Actin filaments and microtubules in the preprophase band and phragmoplast of tobacco cells. Protoplasma **140**, 151-221 (1987).

(24) Lloyd, C. W. and J. A. Traas: The role of F-actin in determining the division plane of carrot suspension cells. Drug studies. Development **102**, 211-221 (1988).

(25) Franke, W. W., W. Herth, W. J. van der Woude and D. J. Morre: Tube and filamentous structures in pollen tubes: possible involvement as guide elements in protoplasmic streaming and vectorial migration of secretory vesicles. Planta **105**, 317-341 (1972).

(26) Herth. W., W. W. Franke and W. J. van der Woude: Cytochalasin stops tip growth in plants. Naturwissenschaften **59**, 38a (1972).

(27) Lancelle, S. A. and P. K. Hepler: Cytochalasin induced ultrastructural alterations in *Nicotiana* pollen tubes. Protoplasma (suppl. **2**), 65-75 (1988).

(28) Mascarenhas J. P. and J. Lafountain: Protoplasmic streaming, cytochalasin B, and growth of the pollen tube. Tissue Cell **4**, 11-14 (1972).

(29) Picton J. M. and M. W. Steer: Determination of secretory vesicle production rates by dictyosomes in pollen tubes of *Tradescantia* using cytochalasin D. J. Cell Sci. **49**, 261-272 (1981).

(30) Tang X., S. A. Lancelle and P. K. Hepler: Fluorescence microscopic localization of actin in pollen tubes: Comparison of actin antibody and phalloidin staining. Cell Motility Cytoskel. **12**, 216-224 (1989).

(31) Derksen, J. and J. A. Traas: Growth of tobacco pollen tubes *in vitro*; effects of drug interference with the cytoskeleton. Proc. 8th Int. Symp. Sexual Reproduction in Seed Plants, Ferns and Mosses, 20-24 August 1984. Willemse, M. T. M., van Went, J. L., eds. Pudoc, Wageningen, The Netherlands. pp. 64-70 (1984).

(32) Heslop-Harrison, J., Y. Heslop-Harrison, M. Cresti, A. Tiezzi and A. Moscatelli: Cytoskeletal elements, cell shaping and movement in the angiosperm pollen tube. J. Cell Sci. **91**, 49-60 (1988).

(33) Terasaka O. and R. Tanaka: Absence of the nucleolar constriction in the division of generative nucleus in the pollen of some angiosperms. Cytologia **39**, 97-106 (1974).

(34) Kadota A. and M. Wada: Circular arrangement of cortical F-actin around the subapical region of a tip-growing fern protonemal cell. Plant Cell Physiol. **30**, 1183-1186 (1989).

(35) Murata, T., A. Kadota, T. Hogetsu and M. Wada: Circular arrangement of cortical microtubules around the subapical part of a tip-growing fern protonema. Protoplasma **141**, 135-138 (1987).

(36) Murata, T. and M. Wada: Organization of cortical microtubules and microfibril deposition in response to blue-light-induced apical swelling in a tip-growing *Adiantum* protonema cell. Planta **178**, 334-341 (1989).

(37) Rutten, T. L. M. and J. Derksen: Organization of actin filaments in regenerating and outgrowing subprotoplasts from pollen tubes of *Nicotiana tabacum* L. Planta **180**, 471-479 (1990).

ヌマムラサキツユクサの花粉分化過程におけるアクチンの動態

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ヌマムラサキツユクサの花粉粒形成と花粉管の発芽，伸長過程におけるアクチンの動態をローダミン・ファロイジン法により解析した．四分子から分裂前期までの小胞子では，繊維状アクチン（F-アクチン）と無構築のアクチンが細胞皮層部または核周辺に少量存在する．F-アクチンは中期には消失するが，後期の終わりから終期において生殖細胞側に湾曲して発達する隔膜形成体内に出現し，隔膜形成体の解体とともに消失する．小胞子分裂後，F-アクチンとF-アクチン束が栄養細胞の核の両側部位を中心に形成をはじめ，細胞全域に大量で複雑なネットワーク状に発達する．成熟花粉粒では，これらのアクチンは著しく細い繊維状へと形状を変え，さらに，花粉管へ移入後，花粉管の成長に応じて太さの異なる繊維束へと再構築される．生殖細胞および精細胞にはF-アクチンは検出されない．小胞子および花粉粒をアクチン重合阻害剤であるサイトカラシンBにより処理すると，花粉管の発芽，伸長は完全に阻害されるが，小胞子細胞の中間期における2回の核移動，隔膜形成体の湾曲した発達および生殖細胞の伸長は阻害されない．以上より，花粉粒内に発達したF-アクチンは主として花粉管の発芽，伸長のために機能することが明らかになった．

本研究により解明された花粉発生過程におけるアクチンの動態を，すでに報告した微小管の動態と比較し，両者の分布と機能に関する明瞭な差異について論議する．