Cell Motility and the Cytoskeleton 65: 553–562 (2008)

Dynamic Rearrangement of F-Actin Organization Triggered by Host-Specific Plant Signal is Linked to Morphogenesis of *Aphanomyces cochlioides* Zoospores

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Cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone), a root releasing host-specific plant signal triggers chemotaxis and subsequent morphological changes in pathogenic *Aphanomyces cochlioides* zoospores before host penetration. The present study illustrates time-course changing patterns of cytoskeletal filamentous actin (F-actin) organization in the zoospores of *A. cochlioides* during rapid morphological changes (encystment and germination) after exposure to cochliophilin A. Confocal laser scanning microscopic analysis revealed that F-actin microfilaments remained concentrated at ventral groove and diffusely distributed in peripheral cytoplasm of the zoospore. These microfilaments dramatically rearranged and changed into granular F-actin plaques interconnected with fine arrays during encystment. A large patch of actin arrays accumulated at one pole of the cystospores just before germination. Then the actin plaques moved to the emerging germ tube where a distinct cap of microfilaments was seen at the tip of the emerging hypha. Zoospores treated with an inhibitor of F-actin polymerization, latrunculin B or motility halting and regeneration inducing compound nicotinamide, displayed different patterns of F-actin in both zoospores and cystospores than those obtained by the induction of cochliophilin A. Collectively, these results indicate that the host-specific plant signal cochliophilin A triggers a dynamic polymerization/depolymerization of F-actin in pathogenic *A. cochlioides* zoospores during early events of plant-peronosporomycete interactions. Cell Motil. Cytoskeleton 65: 553–562, 2008.

Key words: host-specific plant signal; actin polymerization; zoospores; encystment

INTRODUCTION

Actin microfilaments associated with the plasma membrane are important for generating cell-surface specialization, and provide the driving force for remodeling cell morphology or altering cell behavior when the environment is modified [Carreno et al., 2004; Revenu et al., 2004]. This phenomenon is achieved through a tight coupling between cell structure and signal transduction, a process that is modulated by the regulation of actin binding proteins [Brembu et al., 2004; Revenu et al., 2004]. Several lines of evidences suggest that actin plays an important role during differentiation and morphogenesis of the cell [Miller et al., 1999; Hülskamp, 2004; Panteris et al., 2007]. Proper actin organization is

Contract grant sponsor: Japan Society for the Promotion of Science (JSPS), Japan.

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Received 4 October 2007; Revised 18 February 2008; Accepted 12 March 2008

Published online 15 April 2008 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/cm.20281

essential for normal establishment of polarity in many cells including the embryos of the brown algae [Quatrano, 1973; Brawley and Robinson, 1985], polar growth of fungal and Peronosporomycete hyphae [Heath et al., 2000; Riemann et al., 2002] and normal morphogenesis and polar growth of trichomes and root hairs in plants [Miller et al., 1999; Szymanski et al., 1999; Hülskamp, 2004]. Heath et al. [2000] observed that plasma membrane-adjacent F-actin is essential for both polarization and hyphal tip morphogenesis in *Saprolegnia ferax* (Peronosporomycete) and *Neurospora crassa* (fungus). The same study also revealed that actin plays vital role in hyphal polarization and tip morphogenesis rather than microtubules.

Most of the Peronosporomycetes genera such as *Phytophthora, Pythium, Plasmopara, Aphanomyces,* that are phylogenetically distant from fungi, generate characteristic bi-flagellated motile zoospores, are an important means of pathogen distribution and infection of the plants [Agrios, 1997; Judelson and Blanco, 2005]. Zoospores of these phytopathogens precisely locate their hosts guided by the host-specific plant signals followed by a series of morphological changes (encystment and germination) before penetrating the host tissues [Islam et al., 2003]. The host-specific plant signals involved with recognition and pre-infectional development of some zoosporic pathogens have been identified [Horio et al., 1992; Islam and Tahara, 2001, 2005; Islam et al., 2003]. For example, a host-specific signal, cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone) has been identified in the roots and root exudates of the host plants [Horio et al., 1992; Wen et al., 2006] of *A. cochlioides* which triggers chemotaxis and developmental transitions (encystment and germination) of the spores required for successful infection [Islam et al., 2003; Islam and Tahara, 2005]. However, no information is available on how these dramatic morphological changes are occurred by the induction of host-specific plant signal within 30 min of zoospore landing on the host surface [Islam et al., 2003]. As actin acts as a driving force for remodeling cell morphology, the hypothesis of this study is that chemotaxis and subsequent differentiation of zoospores triggered by the host-specific plant signal cochliophilin A is linked to dynamic polymerization/depolymerization of cytoskeletal F-actin in the developing cells of *A. cochlioides*. As a host-free system to visualize chemotaxis and subsequent morphological changes of zoospores to hyphal form, 'particle method' with Chromosorb W AW particles (60–80 mesh) has been used as an excellent dummy of the host roots [Horio et al., 1992; Islam et al., 2003]. This method allowed studying the organization of F-actin in motile zoospores and their dynamic changes that occur during interaction with cochliophilin A by a time-course study using rhodamine-conjugated phalloidin (RP) and observed under a confocal laser scanning microscopy.

A simple compound nicotinamide has been isolated from the roots of nonhost *Amaranthus gangeticus* which induces rapid encystment of *A. cochlioides* zoospores and then triggers regeneration zoospores instead of germinating the cystospores [Islam et al., 2004]. To elucidate the role of F-actin in motility and differentiation processes of zoospore, a potent inhibitor of actin polymerization, latrunculin B [Spector et al., 1983] and nicotinamide were also included in this study. This study revealed that a dynamic changes of F-actin organization occurred during differentiation of *A. cochlioides* zoospores by host and nonhost secondary metabolites and a proper F-actin pattern is necessary for motility of the zoospore. This report describes the results of dynamic polymerization and depolymerization of F-actin in pathogenic zoospores of *A. cochlioides* during induction to morphological changes by a host-specific plant signal, cochliophilin A and discusses findings in relation to the chemical ecological point of view.

MATERIALS AND METHODS

Culture of *A. cochlioides* and Production of Zoospores

A. cochlioides was provided by Prof. R. Yokosawa, Health Science University of Hokkaido, Japan, previously isolated from the soil of sugar beet field and was grown on corn meal agar (17 g/l, Difco Laboratories, Sparks, MD) in the glass petri dish (9 cm i.d.). The zoospores were produced according to the protocol described earlier [Horio et al., 1992; Islam and Tahara, 2001].

Confocal Laser-Scanning Microscopy

The long term effects of F-actin disruption in zoospores, cystospores and germlings were investigated by directly adding appropriate concentrations of latrunculin B (Sigma-Aldrich, St. Louis, MO) (dissolved in dimethyl sulfoxide-DMSO) (Wako Pure Chemical Industries, Osaka, Japan) to zoospore suspension for a set time, after which the corresponding morphology and F-actin organization in the spores were examined. The concentration of DMSO maintained at less than 1% in zoospore suspensions had no effect on motility and F-actin organization in zoospores. The effect of nicotinamide was investigated by homogeneous solution method [Islam et al., 2004], while cochliophilin A was applied through Chromosorb W AW particles coated with 10^{-6} M compound dissolved in EtOAc [Islam et al., 2003]. The time-course changes of F-actin organization in zoospores and cystospores were visualized following the

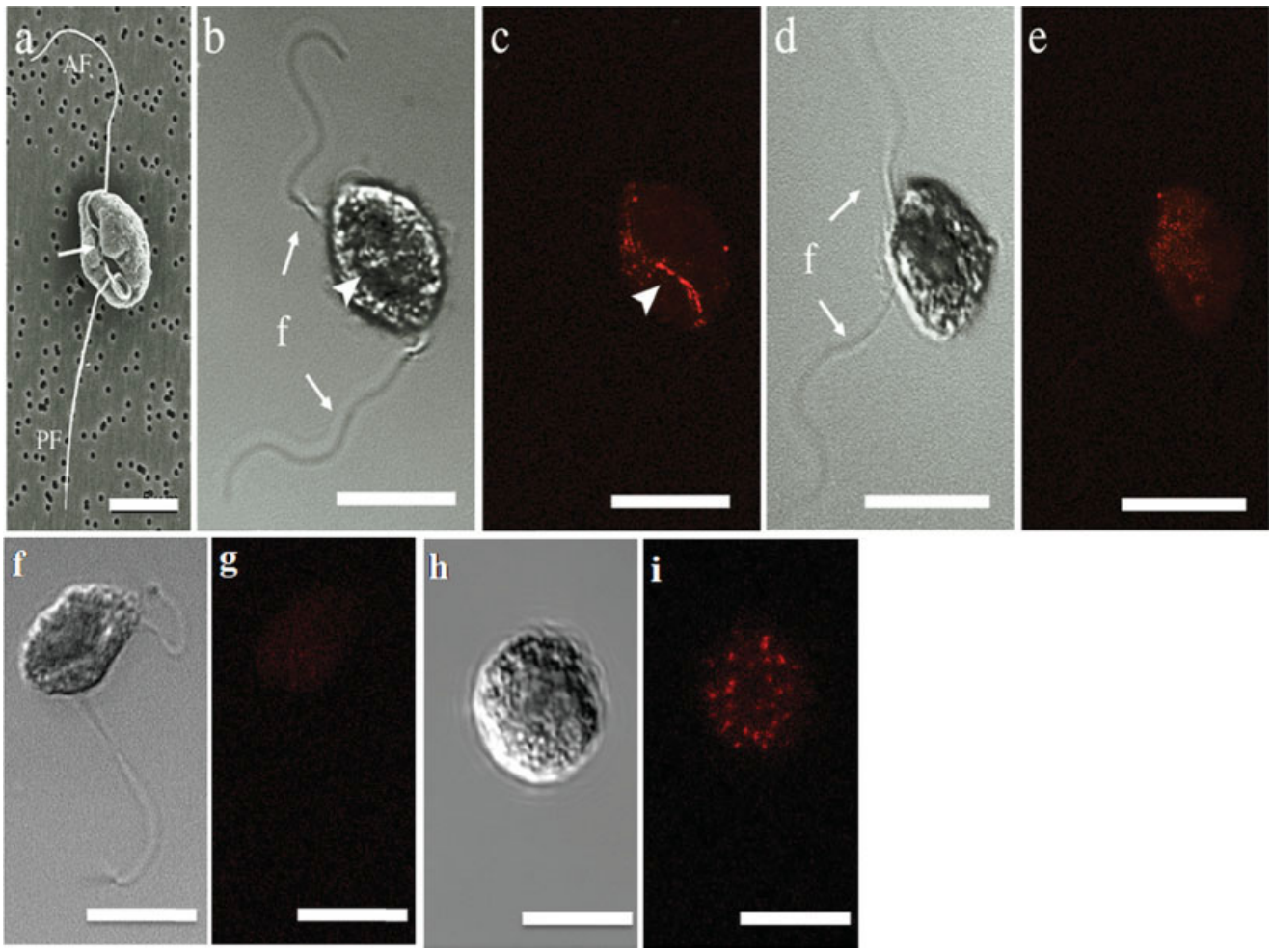

Fig. 1. Morphology and organization of F-actin in *A. cochlioides* zoospores. (**a**) A scanning electron micrograph of glutaraldehyde fixed zoospore. The zoospore has two flagella (AF, anterior flagellum; PF, posterior flagellum) connected to the body at a conspicuous ventral groove (arrow). (**b**, **d**, **f** and **h**) Differential interference contrast (DIC) images of c, e, g and i, respectively (f, flagella). (**c**) A confocal image of actin organization in phalloidin-stained zoospore (control). Fine actin arrays concentrated at the ventral groove region (arrowhead) as well as uniform distribution to the peripheral cytoplasm. (**e**) Disruption of F-actin by latrunculin B for 1 min at the dose of 1 μg/ml. (**g**) Organization of F-actin in nicotinamide (1×10^{-6} M) induced halted zoospore. This zoospore was fixed with paraformaldehyde just 30 s after addition of nicotinamide into zoospore suspension. No F-actin patch is seen at the ventral groove region and the distribution of the fine arrays is not uniform throughout the cell periphery. (**i**) Very defused plaque-form of F-actin in a nicotinamide-induced cystospore. Each treatment was replicated three times and at least 100 spores were observed in each time, and the representative images were shown. White bars in a–i, 5 μm.

method described previously [Heath et al., 2000]. Briefly, 10 μl of appropriate concentrations of latrunculin B (0, 0.25, 0.5, 1, 2, and 10 μg/ml) or nicotinamide solution (1×10^{-6} M) or 3-4 Chromosorb W AW particle coated with 1×10^{-6} M cochliophilin A was added to 90 μl of spore suspension on a glass slide and allowed to sit for 0, 1, 5, 10, 15, 20, 30, 40, 60, and 90 min in a humid chamber at room temperature (23°C). Then the specimens were fixed with 6% paraformaldehyde (Wako Pure Chemical Industries) in 60 mM 1,4-piperazinediethanesulfonic acid (PIPES) buffer (pH 7.0, Sigma-Aldrich), with 100 μM MBS (*m*-maleimidobenzoyl *N*-hydroxysuccinimide ester, Pierce Chemical, Rockford, IL) for 30 min. Specimens were then rinsed in PIPES buffer alone, stained for 30 min in 0.66 μM RP (Molecular Probes, Eugene, OR) in 60 mM Pipes buffer (pH 7.0), rinsed in buffer, and then mounted in 50% glycerol with 0.1% *p*-phenylenediamine. The specimens stained with RP were observed by a Carl Zeiss confocal laser scanning microscope (CLSM) LSM410 (Carl Zeiss, Germany). Image capture and arrangement were performed as previously described [Deora et al., 2006]. This experiment was repeated three times and each treatment was also replicated at least three times. The representative images are presented. Nicotinamide (Sigma-Aldrich), latrunculin B (Sigma-Aldrich), RP (Molecular

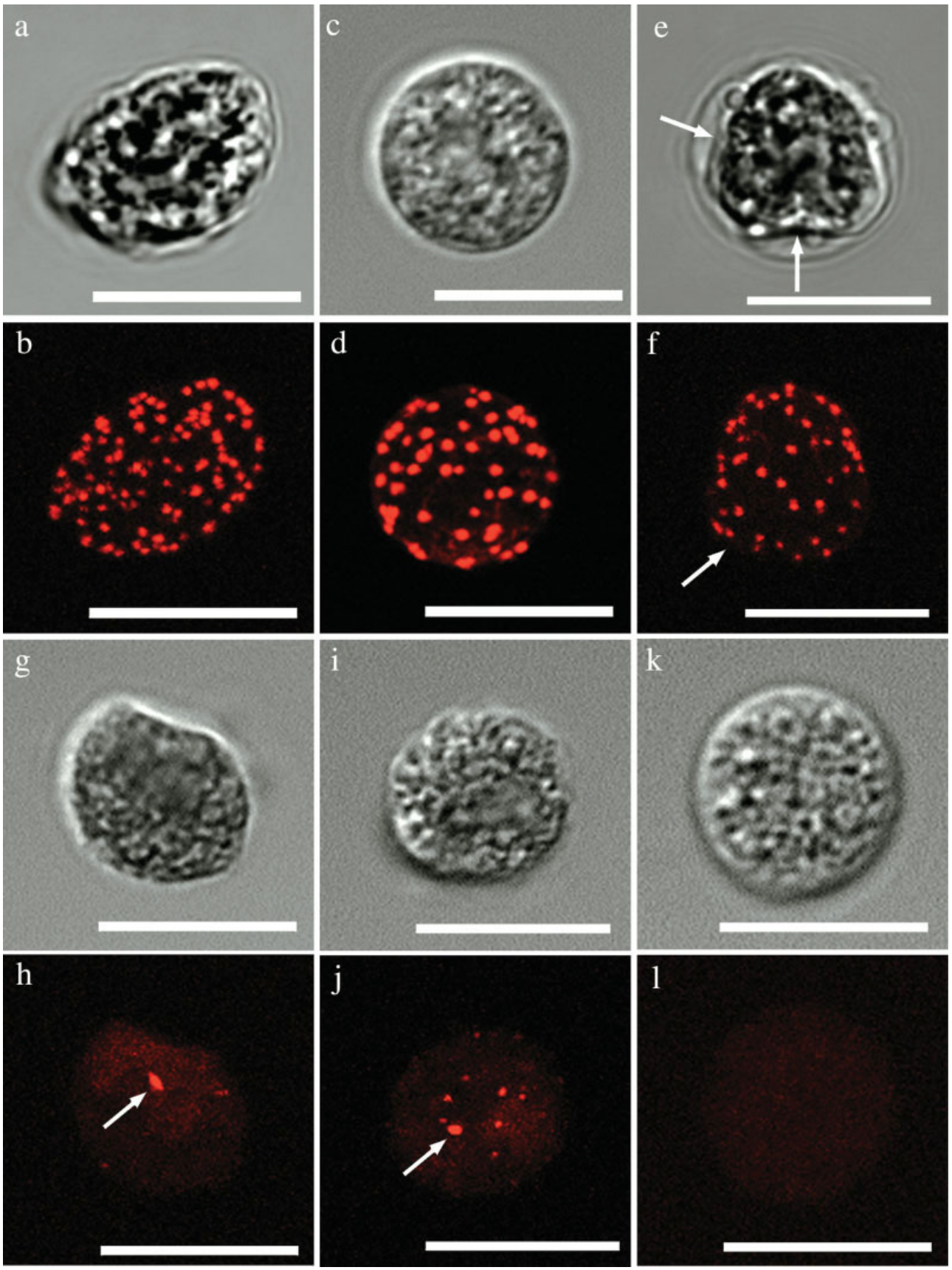

Figure 2.

Probes) were purchased and cochliophilin A was synthesized in the laboratory [Horio et al., 1992].

Transmission Electron Microscopy

The zoospores were induced to encystment and germination by cochliophilin A using Chromosorb W AW particles taken on a small glass Petri dish (3 cm i.d.) for 15 and 30 min. The resulted cystospores and germlings were fixed with 3% glutaraldehyde (TAAB, Birkshire, UK) and rest of the procedures for transmission electron microscopy (TEM) study were carried out as described previously [Islam et al., 2005; Islam, 2008]. This experiment was replicated three times and the representative images are presented.

RESULTS

F-Actin Microfilaments in the Motile Zoospores

Motile biflagellated *A. cochlioides* zoospores that were first fixed with 6% buffered paraformaldehyde followed by staining with RP and observed under a CLSM for F-actin organization is shown in Fig. 1b and 1c. In zoospore, F-actin microfilaments remained concentrated at the ventral groove region where two flagella are connected to the spore and diffusely distributed in the peripheral cytoplasm (Fig. 1c, arrow). Zoospores halted by latrunculin B (1 μg/ml) gave much diffused actin fluorescence compared to control (Fig. 1e). However, zoospores had fixed with paraformaldehyde immediately after halted (Fig. 1g) by nicotinamide showed completely different organization of actin in the cells. In both cases, no clear patch of actin arrays were seen in the ventral grove region as observed in all control zoospores.

Actin Microfilaments Changed Into Granular Plaques During Encystment of Zoospores Triggered by a Host-Specific Plant Signal Cochliophilin A

The F-actin microfilaments were dramatically rearranged and changed into granular plaques during encystment of zoospores (Figs. 2b and 2d). An encysting spore which was fixed with glutaraldehyde just 5 min after exposure of a zoospore to the host-specific compound cochliophilin A is showing abundance of actin plaques in Fig. 2b. This spore is yet to completely change into a round-shaped cystospore. In mature cystospores (fixed 20 min after interaction with cochliophilin A), the plaques distributed uniformly and inter-connected with actin microfilaments in a continuous network throughout the cell (Fig. 2d). Optical sectioning at the middle of a cystospore showed that these arrays and plaques were essentially planer, adjacent to the plasma membrane, with no visible elevations in the cell surface (Figs. 3a and 3b). The size of the actin plaques became the largest in the mature cystospore (Fig. 2d) and then changed slightly into smaller plaques in the germinating cystospores (Fig. 2f) as well as in the growing hyphae (Figs. 4b, 4d, 4f, and 4h). Zoospores induced to encystment by nicotinamide also showed peripheral actin plaques which were relatively small in size than those produced by cochliophilin A (Fig. 1i).

Dynamic Changes of F-Actin Plaques During Germination of Cystospore

When the zoospores of *A. cochlioides* exposed to a Chromosorb particle coated with 1×10^{-6} M cochliophilin A, they became round-shaped cystospores within

Fig. 2. Confocal images (**b**, **d**, **f**, **h**, **j**, and **l**) of three progressive stages of *A. cochlioides* spores induced by host-specific plant signal cochliophilin A showing normal F-actin organization (b, d, and f) and their disruption (h, j, and l) by latrunculin B. DIC images of the respective spores are presented just before their confocal images. **a**, **c**, **e**, **g**, **i**, and **k** are the DIC images of the b, d, f, h, j, and l confocal images, respectively. Aqueous suspension of zoospores (ca. 10^5/ml) was induced to encyst by a host-specific plant signal cochliophilin A (10^{-6} *M*) or latrunculin B dissolved in 1% of DMSO, and allowed to incubate on a slide glass placed in a moistened chamber until set time. Then the samples were fixed with 6% paraformaldehyde followed by actin staining with rhodamine-conjugated phalloidin and observed under a confocal laser-scanning microscope. (b) Smaller plaques of peripheral F-actin inter-connected by short microfilaments in an encysting zoospore (oval-shaped). This zoospore was induced to encyst by a host-specific plant signal cochliophilin A and fixed with 6% paraformaldehyde 15 min after interaction and then stained with rhodamine-conjugated phalloidin. (d) Larger plaques of peripheral F-actin inter-connected by microfilaments in a mature round-shaped cystospore. This cystospore was fixed with paraformaldehyde after 20 min interaction with cochliophilin A. (f) F-actin plaques became slightly smaller 25 min after treatment (just before germ tube emergence). Arrow indicates a slight bulge formation depicting the site of the germ tube emergence. The respective DIC image (e) shows contraction in cellular materials, a characteristic phenomenon usually observed before germination of a cystospore. (h) Disruption of F-actin organization in an encysting zoospore by latrunculin B at 1 μg/ml for 10 min. Latrunculin B ceases motility of the zoospore and then caused encystment. Formation of actin plaques during encystment of zoospores is blocked, and only one oval shaped large plaque is seen (arrow). (j) An actin plaque associated with very few smaller ones in a cystospore produced by latrunculin B (1 μg/ml for 15 min). Number of plaques is very lower compared to control (d). (l) Complete disruption of F-actin plaques in a cystospore exposed to a higher dose of latrunculin B (2 μg/ml) for 30 min. Each treatment was replicated three times and at least 50 spores were observed in each time, and the representative images were shown. White bars in a–l, 10 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

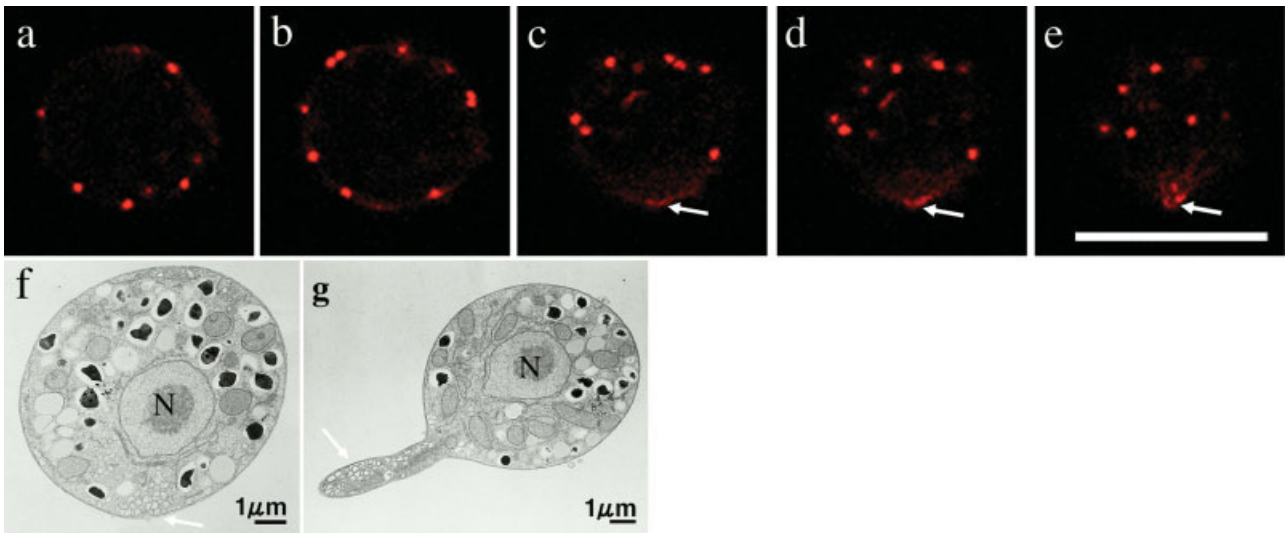

Fig. 3. CLSM optical sections (**a**–**e**) of a germinating cystospore and two TEM micrographs showing sites of vesicle accumulation in the spores just prior (**f**) and after (**g**) germ tube emergence. (a, b) Peripheral distribution of F-actin plaques in two optical sections that taken at the middle of the cell. Arrows in c–e indicate F-actin cap showing the site of germ tube emergence. (f) Accumulation of vesicles (arrow) at one pole of the cystospore prior to the formation of germ tube. (g) Vesicle accumulation in the tip of the growing germ tube. N, nucleus. White bar in e, 10 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

15 min and then germinate to form hyphal germ tubes (within 30 min). To investigate the organization of F-actin in the germinating cystospores, the cytospores were fixed before (20 min after treatment) and after germination (30 min after treatment). Fine microfilaments of F-actin were accumulated in a large patch at one pole of the cystospore as seen in Figs. 3c–3e. This specific polar accumulation of actin seems to be one of the earliest markers of germ tube formation. Since actin acts as a track of vesicle transport, an investigation by TEM was carried out to see whether vesicles are also accumulated at the pole of the cystospore before germination. As expected, a high accumulation of vesicles at one pole of the cystospore was observed immediately before germination of the cell (Fig. 3f, arrow).

To observe the dynamics of F-actin during transformation of a unicellular cystospore to a multicellular hypha, germinated cystospores were fixed with paraformaldehyde at different time intervals (30, 40, 60, and 90 min after treatment). The arrangements of F-actin again changed remarkably during formation (Figs. 3c–3e) and expansion of the germ tube (Fig. 4b) from a cystospore. The actin plaques gradually move from the cell body to the emerging germ tube and were interconnected by longer microfilaments (Figs. 4b, 4d, and 4f). At the tip of the germ tube, a distinct cap of actin microfilaments was visible (Fig. 4b, arrow). This actin cap was also observed when a new tip was formed in the hyphal germ tube (Fig. 4h, arrow) indicating its requirement in initiation and growth of the tip. No F-actin cytoskeleton was observed in the empty cyst-coat (ghost) after cellular materials completely moved to the growing hyphal germ tube (Figs. 4d, 4f, and 4h). A TEM section of a germinated cystospore also confirmed the accumulation of vesicles in the bulge or tip of the growing germ tube (Fig. 3g, arrow).

Zoospores immediately halted (100%) and changed into round-shaped cystospores due to an exposure to nicotinamide at 1×10^{-8} to 1×10^{-6} M concentrations. Instead of germination, these cystospores regenerated zoospores (ca. 80–90%) leaving their cyst-coats within 3 h after encystment [Islam et al., 2004]. To investigate whether nicotinamide disrupts normal F-actin patterns in halted zoospores and regenerating cystospores or daughter zoospores (third generation), time-course changes of F-actin in nicotinamide treated zoospores was visualized. Nicotinamide halted zoospores showed diffused arrays of F-actin compared to control motile zoospores (Fig. 1g). The F-actin became granular plaques in the cystospores induced by nicotinamide (Fig. 1i) but the size of the plaques was smaller in the cystospores (Fig. 1i) than those observed for host-specific cochliophilin A (Fig. 2d). However, the changing pattern of F-actin did not vary by higher doses from the minimum effective dose of nicotinamide. Interestingly, the F-actin plaques dramatically disappeared when a nicotinamide-induced cystospore regenerated zoospore leaving a cyst-coat (ghost) (data not shown). It clearly shows that plaque form of F-actin is absent in zoospores. The ghost also did not contain any F-actin population indicating that cell wall is free from the F-actin cytoskeleton.

Lantrunculin B Halts Motility of Zoospores and Inhibits Germination of Cystospores by Disrupting F-Actin

To get precise evidence of the participation of cytoskeletal F-actin in the process of encystment of zoospores and subsequent germination of the cystospores, the effect of latrunculin B, a potent inhibitor of F-actin assembly on zoospores and germinating or germinated cystospores was examined (Figs. 4j, 4l, and 4n). Cochliophilin A induced cystospores germinated (100%) to form germ tubes within 30 min after encystment. In contrast, drug latrunculin B rapidly accelerated the transition from free zoospores to cystospores at the range of 0.5–10 μg/ml. The motility of the zoospores gradually ceased and almost all zoospores changed into round-shaped cystospores within 30–60 min. CLSM observation revealed that latrunculin B disrupted F-actin microfilaments in the zoospore (Fig. 1e) and both arrays and plaque form of F-actin in the cystospores (Figs. 2h, 2j, and 2l) and germ tubes (Figs. 4j, 4l, 4n, and 4p) in a dose dependent manner. It clearly disrupted F-actin wires at low concentration but at higher concentrations, it also affected both wires and plaque form of F-actin in all types of *A. cochlioides* spores. Interestingly, germination of the cystospores was completely blocked in the presence of latrunculin B at 1 μg/ml or higher concentrations until 60 min. However, this compound completely eliminated F-actin and caused lysis of zoospores at 10 μg/ml (Figs. 4o and 4p).

In order to demonstrate the importance of the radial F-actin arrays for initiating polar growth, cochliophilin A induced cystospores were incubated in the presence of 0, 0.25, 0.5, 1.0, and 2.0 μg/ml latrunculin B. By 60 min, almost 100% cysts had germinated and produced normal germ tubes (Fig. 4b) in cochliophilin A treated dish, while germination in the presence of latrunculin B was blocked in a dose dependent manner (Fig. 4j). Some cysts apparently achieved some polarization (Fig. 4j), resulting in irregular protrusions and did not proceed to the formation of normal germ tubes until 60 min after the treatment (Fig. 4l). When cochliophilin A-induced cystospores were exposed to the latrunculin B, the polar growth of the germ tubes diminished and tips became swollen (Figs. 4l and 4n).

DISCUSSION

Cytoskeleton, composed largely of actin filaments, is the internal framework of a cell. It includes microtubes, microfilaments and middling fibers, and is the base structure for cell motility, cell morphology and transmembrane signal transduction [Carlier and Pantaloni, 1997]. Cellular motility driven by assembly and disassembly of actin filaments have been observed in many motile cells including peronosporomycete zoospores [Theriot and Mitchison, 1991; Heath and Harold, 1992; Pollard and Borisy, 2003]. However, the role of actin organization in motile zoospores of peronosporomycete phytopathogens and their changes during differentiation to the filamentous hyphae while interacting with host plants or host-specific signaling molecules has not been elucidated.

The present study has revealed that the organization and population of F-actin in *A cochlioides* zoospores is dynamically rearranged during the developmental transitions (cystospores and germination to give hyphae) triggered by a host-specific plant signal cochliophilin A (Figs. 1, 2, and 4). In addition, zoospores those were halted by a nonhost signaling molecule, nicotinamide showed different actin organization in their cells compared to the actin organization of the motile zoospores (Fig. 1g). These findings suggest that an appropriate F-actin organization might be required for the motility of zoospores. This study confirmed that actin remains only in microfilament form in the motile zoospores. It appears that actin in microfilament form impart the flexibility to the zoospores that facilitates them in changing their shapes during swimming and docking on the host surface [Islam et al., 2002].

When the zoospores were induced to encystment by host-specific compound, cochliophilin A, actin microfilaments were dramatically changed into granular plaques interconnected with fine microfilaments. Similar phenomenon of plaque-form actins in cystospores has been found in other peronosporomycetes [Heath et al., 2000; Riemann et al., 2002]. During initiation of germination of a cystospore or a branch on a germ tube, the germination points were rich in microfilaments, but the vicinal regions to the germination points lacked plaques population. This clearly reflects that plaques are depolymerized into microfilaments in a certain region of the cell to facilitate germination. TEM analyses revealed that vesicles are accumulated at the site of cystospore from where germ tubes are emerged. The accumulation of vesicles at the site of germ tube emergence and the tip of the growing germ tubes might be related to the transport of cell wall materials and enzymes required for the development of the wall material at the tip [Heath, 1995]. The observed colocalization of cytoskeletal F-actin (Figs. 3c–3e) and vesicles (Fig. 3f) at the site of germ tube emergence indicates that vesicles are transported along the F-actin cytoskeleton. Such actin caps have also been observed in many tip growing systems including the hyphae of other Peronosporomycetes [Heath et al., 2000; Hülskamp, 2004; Islam, 2008]. In plant, these F-actin caps remain associated with tips of emerging and growing root hairs [Baluška et al., 2000]. The inhibition of motility and germination of cystospores was found to

be correlated with the disruption of actin in the cells by F-actin polymerization inhibitor, lantrunculin B. These results clearly indicate that appropriate F-actin organization is important not only for germination of the cystospore but also for the polar growth of the newly formed hypha of *A. cochlioides* [Islam, 2008]. The observed dis-

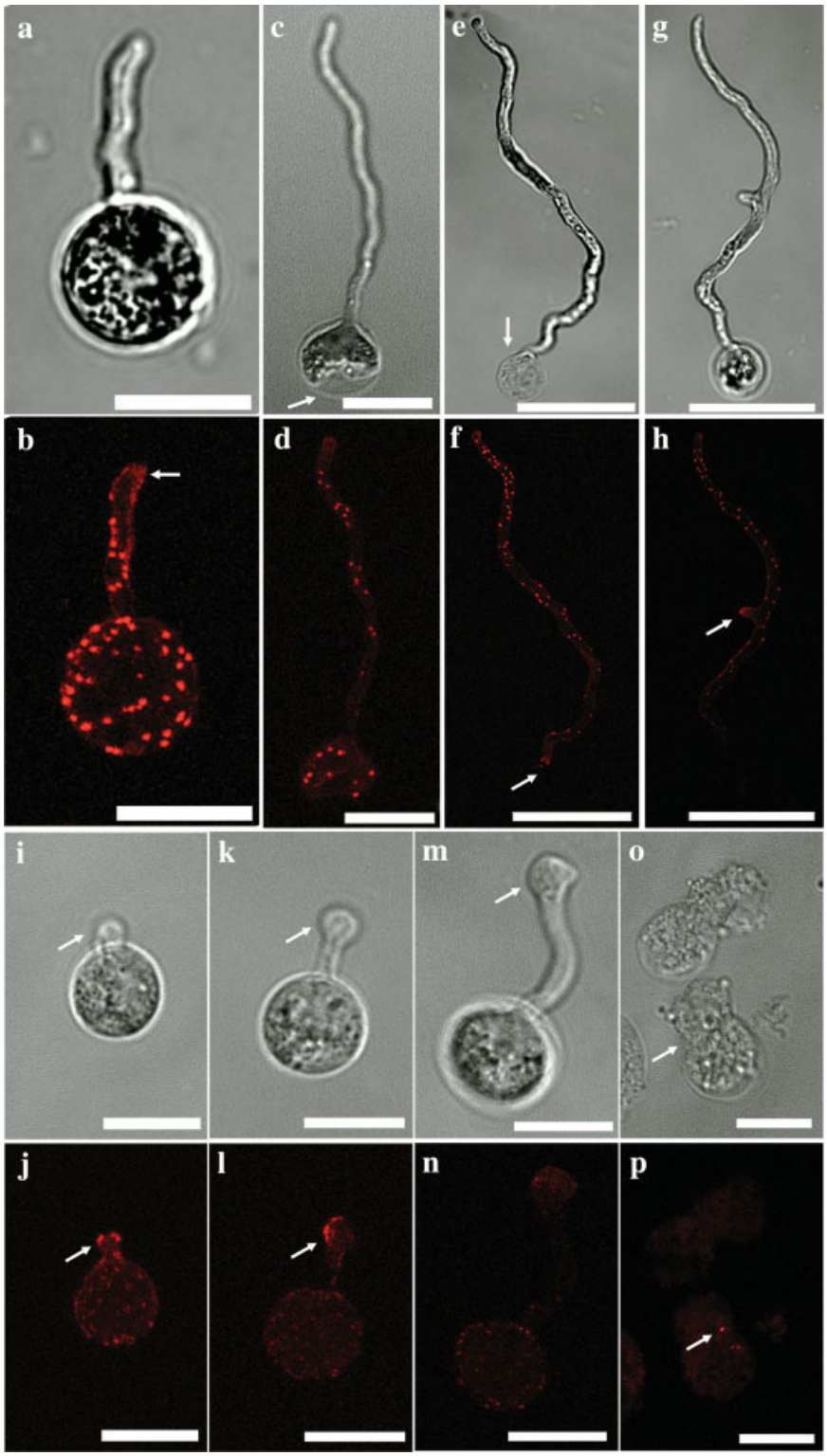

Figure 4.

tribution of actin during the individual stages indicates how the dissolution and repolymerization of this structure could work.

Parallel CLSM and TEM studies revealed that rearrangement of F-actin organization is directly linked to the dynamic morphological changes in early development of the damping-off pathogen *A. cochlioides*. The precise arrangement of F-actin and their dynamic reorganization during developmental transitions of zoospores triggered by a host-specific plant signal demonstrated in this study has not been reported so far, to the best of my knowledge. Although the stage-specific assembly and disassembly of actin plaques were found in other Peronosporomycetes [Jackson and Hardham, 1998; Riemann et al., 2002], this is the first study that illustrates the role of a host-specific plant signal on dynamics of F-actin organization during developmental transition of Peronosporomycete zoospores. The actin plaques in cystospores and other later stages appeared to be the organization centers related to remodeling of cell structure and the transition of cell into a new stage [Riemann et al., 2002].

The actin microfilaments those were either concentrated at the sites of germination of cystospores or at the tips of new hyphal branch were associated with deposition of vesicles (Figs. 3c–3g and 4h). The presence of actin caps (Figs. 3c–3e) and vesicle accumulation at the tip of the growing germ tubes (Fig. 3g) suggest that actin plays a vital role in cystospore germination as well as polar growth and branching of the hyphal germ tube [Islam, 2008]. It is possible that actin polymerization supports the formation and growth of germ tube more directly, by exerting a driving force on the plasma membrane. Borisy and Svitkina [2000] proposed that pollen tube tip growth is a form of cell movement in plants and that F-actin polymerization and depolymerization at the tube tip might be a motive force.

CONCLUSION

The overall considerations of the above findings allows to hypothesize that signaling compounds from host and nonhost plants trigger defined dynamic changes in organization and reorganization of actin in pathogenic zoospores during early events of plant-peronosporomycete interactions. This study for the first time demonstrated that rapid morphological changes of zoospores of a peronosporomycete phytopathogen *A. cochlioides* resulting to hyphae is triggered by a host-specific plant signal cochliophilin A. This phenomenon is associated with dynamic organizational changes of filamentous actin in the developing cells. This finding advances our understanding on the basic cytology of the differentiation of the zoospores of a phytopathogenic Peronosporomycete by a host-specific plant signal that guide them to find the host by chemotaxis [Islam et al., 2003, 2004]. A future challenge will be the elucidation of the signal transduction pathways of F-actin polymerization by the host-specific plant signals. Relatively simple zoospores production protocol and its synchronous developmental changes to cystospores and subsequent transition to the hyphal form triggered by a host-specific plant signal, cochliophilin A in host-free conditions would allow to use *A. cochlioides* as an excellent model system to study all aspects of cell differentiation at the single-cell level. This may include the choice of cell fate, developmental control of the cell cycle, cell polarity and the control of cell shape.

Fig. 4. CLSM images (**b**, **d**, **f**, **h**, **j**, **l**, **n**, **p**) and corresponding DIC micrographs (**a**, **c**, **e**, **g**, **i**, **k**, **m**, **o**) showing normal (cochliophilin A induced) (b, d, f, h) and disrupted (by latrunculin B) (j, l, n, p) F-actin organization in germinated cystospores. Zoospores (ca. 10^5/ml) were induced to encyst by cochliophilin A (10^{-6} M) or appropriate concentration of latrunculin B dissolved in 1% dimethyl sulfoxide (DMSO) and allowed to incubate on a slide glass until set time. Then the samples were fixed with 6% paraformaldehyde followed by actin staining with rhodamine-conjugated phalloidin and observed under a confocal laser-scanning microscope. (b) F-actin distribution in a germinated cystospore (60 min after encystment by cochliophilin A). Plaques are interconnected by fine arrays and distributed throughout the cystospore and the germ tube except its tip. An arrow indicates cap of F-actin arrays in the tip of the germ tube. (c) A position of the cystospore (opposite to the germ tube site) becomes empty (arrow) due to the migration of the cellular materials to the growing germ tube (80 min after encystment by cochliophilin A). (d) Distribution of F-actin plaques and arrays in the growing germ tube. No actin organization in the respective empty portion of the cystospore (arrow in c). (e) Cellular materials completely moved from the cystospore to the growing germ tube (90 min). No F-actin fluorescence in the empty cyst-coat (arrow in f). (h) F-actin cap in the tip of a new branch (arrow). (j) Disruption of F-actin in latrunculin B (1 μg/ml for 60 min) treated spore. A protrusion/bulge (arrow in i) is developed but formation of normal germ tube (see a and b in the same duration in control) was blocked. Plaques are severely reduced in size. The polar growth of this protrusion was not advanced until 90 min. (l) Latrunculin B (1 μg/ml) completely eliminated plaque form of F-actin in a freshly germinated cystospore exposed for 10 min. The cystospore was germinated in sterile water after 40 min of encystment by cochliophilin A and then exposed to latrunculin B. The tip of the germ tube becomes swollen and its polar growth was completely stunted. (n) Disruption of F-actin in a germinated spore, which was exposed to latrunculin B for 20 min after a certain elongation of germ tube (60 min interaction with cochliophilin A). The polar growth of the germ tube was stunted until 90 min of exposure and the tip becomes deformed and swollen. (o, p) Complete disruption of F-actin and lysis of zoospores at higher concentration (10 μg/ml) of latrunculin B. White bars in a, b, and i–p, 5 μm; and c–h, 10 μm.

ACKNOWLEDGMENTS

The author is very thankful to Prof. S. Tahara and Prof. Y. Hashidoko, Graduate School of Agriculture, Hokkaido University, Japan, for their enormous support and encouragement during this study. Special thanks are due to Prof. R. Yokosawa, Health Science University of Hokkaido, Japan, for kindly providing *A. cochlioides* (AC-5) and Ms. Y. Aoyama and Mr. T. Ito, Electron Microscopic Laboratory, Hokkaido University, for technical assistance during microscopic studies. The author expresses sincere thanks to Dr. M. Rahman (NCSU Dept. Plant Pathology) for linguistic editing.

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