An Ultrastructural Study of Photo-induced Conidiogenesis and Dedifferentiation in *Alternaria solani*

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Ultrastructure of conidiophores has been studied in *Alternaria solani*, pathogenic fungus of potato early blight, in order to elucidate the difference of cytoplasmic structures between the fertile conidiophore and the defifferentiating conidiophore induced by blue light irradiation. In the fertile conidiophore the electron transparent structure developed at the apex and it seems to restrict the cytoplasm to contact with the cell wall of apical dome only at the point of the pore. Concomitantly with the development of the electron transparent structure, a large vacuole developed in the apical cell of the fertile conidiophore. The vacuole is presumed to provide the turgor pressure for protrusion of cytoplasm through the pore to form a conidium initial. Blue light exposure inhibited both the electron transparent structure at the apex and the development of vacuole in the cytoplasm, thus it reverted the fertile conidiophore to the vegetative hypha.

Introduction

In many fungi, conidium formation is induced by light (Marsh et al., 1959). Effective wave region for sporulation induction differs with the species of fungi and it extends from ultraviolet to far red (Tan, 1978). However, most fungi are induced to sporulate by ultraviolet and blue light wave regions.

Although certain fungi require light to form conidiophores, light also inhibits conidium formation (Aragaki, 1962; Honda, 1968). Inhibition of conidium formation is brought about by defifferentiation of a conidiophore into a vegetative hypha by light (Honda, 1969; Aragaki, 1973), and the effective wave region for inhibition is blue light (Aragaki, 1962). Through the determination of an action spectrum for sporulation inhibition of *Alternaria solani* (Ellis et G. Martin) Sorauer, Honda and Nemoto (1984) revealed that the photoinhibition of sporulation was a typical blue light effect.

Microscopical observation on dedifferentiation of conidiophores was made for *Alternaria tomato* (Cke.) Weber (Aragaki, 1973) and *Helminthosporium oryzae* Breda de
Haan (Honda, 1969). However, ultrastructure of deifferentiation of conidiophores in contrast with that of conidiogenesis is yet to be explored. In this report we observed ultrastructure of an apical cell of conidiophore which was induced to dedifferentiate into a vegetative hypha by blue light exposure and a fertile conidiophore which was to sporulate under darkness.

**Materials and Methods**

**Organism**: A single spore of *Alternaria solani*, isolate A18, was isolated from an early blight lesion of a potato leaf cultivated in an experiment field of Shimane University.

**Culturing**: Single spores from a newly sporulated colony were used as inocula to initiate cultures on vegetable juice agar (VJA, Campbell's V-8 juice 200 ml, CaCO₃ 2 g, agar 17 g, distilled water 800 ml, with the final pH 5.8; 10 ml/60 mm Pyrex Petri dish) and the colonies were incubated under darkness at 25°C for 4 days, at which time the colony was ca. 35 mm in diameter. Aerial mycelia of the colonies were then removed by a rubber spatula and the colonies were rinsed twice with sterilized, distilled water. Surface of these colonies were then dried under darkness at room temperature for 1 hr.

**Irradiation**: Colony margins were marked on the bottom of the Petri dish to locate the region to be sampled. The colonies were then irradiated for 6 hr with near ultraviolet (NUV) radiation from a 20 W black light fluorescent lamp (FL20S BLB, Toshiba Corporation, Tokyo; 310-410 nm, max. 360 nm) to induce conidiophore formation. The lamp was suspended 18 cm above the colonies. The colonies were then transferred to darkness. In order to induce dedifferentiation of conidiophores, the colonies were irradiated for 2 hr with blue light from three 20 W blue-white fluorescent lamps (FL20BF, Toshiba Corporation, Tokyo) suspended 10 cm apart and 18 cm above the colonies. The colonies were covered with a band pass filter (BPB-45, Fuji Photo Film Co., Ltd., Tokyo; transmission range of 400-500 nm with max. 450 nm) to restrict the radiation to the most effective wavelengths to induce dedifferentiation of conidiophores.

**Electron microscopy**: Chunks (1×3 mm) of dense conidiophores were taken from the marked portion of colonies which were incubated under darkness for 6, 8 or 10 hr after NUV irradiation and were fixed at 4°C for 3 hr in 6.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. A vacuum treatment for 15 min allowed better penetration of the fixative. The conidiophores from blue light irradiated colonies were also fixed at the end of 2 or 4 hr darkness after 2 hr blue light irradiation. The material was then washed three times with 0.1 M phosphate buffer, and post fixed for 3 hr with a phosphate-buffered 1% osmium tetroxide solution at pH 7.4. After three time rinses in distilled water, the material was dehydrated in a graded ethanol series followed by 100% propylene oxide for 1.5 hr with several changes. The material was embedded in Spurr's low-viscosity medium (standard medium) (Spurr, 1969). Polymerization of the resin was carried out at room temperature for 1 hr followed by 45°C for 6 hr, 60°C for 12 hr and 70°C for 24 hr.
Sections were cut with a glass knife on a Porter-Blum MT-2B ultramicrotome and collected on Formvar-coated 100-mesh copper grids. They were then stained 3 hr at room temperature with 5% uranyl acetate. The sections were stabilized with a light carbon coating and examined with a Hitachi HU-12A electron microscope working at 75 kV. Fuji Electron-Microscopic Film FG was developed in Fuji Copinal. Fujibro WP FM 3 paper was developed in Fuji Microfine.

**Results**

Conidiophores, induced by 6 hr NUV irradiation, elongated along with the increase in the number of cells during 6 to 8 hr darkness following NUV irradiation. Then conidiophores ceased to elongate and the cell wall at the apex of conidiophore thickened, especially an osmiophilic outer layer of cell wall increased its thickness. The center of apical dome of conidiophore indented slightly, suggesting the location of the pore formation. A portion of vacuole was observed between the apex and a nucleus. Many mitochondria were also found around the vacuole (Fig. 1).

A special feature appeared inside the apical dome. This structure had a low electron density and should be called as an electron transparent structure (ETS). It did not cover entire inside area of apical dome, but there was a cavity at the center through which the cytoplasm reached to cell wall at the apex. At that point the cell wall indented from both sides and became thinner than other portion. Mitochondria converging at the subapical zone had slender shape suggesting preparation for migration through a pore into a conidium initial (Fig. 2).

Myelin figures were often observed on underside of electron transparent structure. Unit membrane constituted myelin figures and the same membrane bounded cytoplasm-side of electron transparent structure. Cytoplasm seems to be pushed up to cell wall through the central cavity of ETS. There were many apical vesicles in the apical zone of cytoplasm where cytoplasm was in contact with cell wall.

Mitochondria converged in the midway between the apex and vacuoles which developed in the middle to basal portion of the cell (Fig. 3). There were several nuclei and they tended to locate at the apical side of the developed vacuolar system. The apex...
Fig. 2. Apical dome of conidiophore showing electron transparent structures (E) with myelin figures (MF). Apical vesicles (A) are observed in the central cavity of electron transparent structure where cytoplasm is in contact with the cell wall. After 8 hr darkness following conidiophore induction by 6 hr NUV irradiation. OL: Osmiophilic outer layer. Scale=0.5 μm

of the conidiophore was rounded and the outer and inner walls, especially the osmiophilic outer layer became thin at the point where the pore for a conidium initial would be formed. In process of time cell wall at the apex rapidly decreased the thickness and the osmiophilic outer layer almost disappeared (Fig. 4). The apical vesicles were seen to move to that point.

Then cell wall came to an end abruptly thus delimiting a somewhat cylindrical pore at the apex. Cytoplasm covered with only plasma membrane was pushed out through the pore to form an initial of conidium (Fig. 5). Electron transparent structure was still apparent around the neck of a conidium initial. The conidium initial did not have cell wall at this moment. Cell wall of conidiophore discontinued with the conidium initial. Cytoplasm being pushed through the pore became round without the cell wall (Fig. 6). Because a plane of section deviated slightly from the central axis of conidiophore, the cytoplasm of conidium initial did not seem to be continuous with that of the apical cell of conidiophore in Fig. 6. Later on the cell wall deposited around a new-born conidium (Fig. 7). A nucleus already migrated into the conidium at this stage of conidium development. Electron transparent structure was still apparent at the apical dome of the conidiophore and it did not show the central cavity, because of slight deviation of a section plane from the central axis of conidiophore. The osmiophilic outer layer of the cell wall was conspicuous at the apex and it contrasted with a more or less thin osmiophilic outer layer in the other portion of the cell wall.
On the contrary to the process of conidium formation under darkness, ETS did not develop in the conidiophore apical cell which was subjected to 2 hr blue light exposure after 6 hr darkness following induction of conidiophore by 6 hr NUV irradiation (Fig. 8). Small vacuoles dispersed along the length of the apical cell. Some vacuoles contained myelin figures. A large vacuolar system did not develop
contour of the fertile conidiophore kept under darkness. Plasma membrane at the apex was not smooth and there were many apical vesicles in the apical zone of cytoplasm. Cell wall of newly elongating apex continued to other basal part of cell which was thick and had an electron-dense outer layer (Fig. 10). Following the sharpening of the apex, the conidiophore grew as a vegetative hypha with smaller diameter. Several nuclei were found in the central part. A part of striated micro-filament bundles was seen in the subapical zone.

in the apical cell which was irradiated by blue light. There was no indication of dedifferentiation at this moment. Two hr later under darkness after 2 hr blue light irradiation, the cell wall at the apex became thinner and electron transparent with a parabolic contour (Fig. 9) sharply contrasting with the hemisphere or flat contour of the fertile conidiophore kept under darkness. Plasma membrane at the apex was not smooth and there were many apical vesicles in the apical zone of cytoplasm. Cell wall of newly elongating apex continued to other basal part of cell which was thick and had an electron-dense outer layer (Fig. 10). Following the sharpening of the apex, the conidiophore grew as a vegetative hypha with smaller diameter. Several nuclei were found in the central part. A part of striated micro-filament bundles was seen in the subapical zone.

Fig. 4. A part of the apex where a pore (P) for a conidium initial will be formed. Cell wall, especially osmiophilic outer layer (OL) at the point almost disappeared. Apical vesicles (A and small arrows) seem to move to the point through the central cavity of the electron transparent structure (E). After 8 hr darkness following conidiophore induction by 6 hr NUV irradiation. Scale=0.2 μm

Fig. 5. A blowing out of the plasma membrane (PM) to form a conidium initial through a pore (P) in the cell wall. Osmiophilic outer layer (OL) prevails almost entire cell wall around the pore. Electron transparent structure (E) is apparent at the neck of the conidium initial. After 8 hr darkness following conidiophore induction by 6 hr NUV irradiation. Scale=0.2 μm
The striated microfilament bundle was specifically observed in the conidiophore which was irradiated by blue light and started to dedifferentiate into a vegetative hypha (Fig. 11). The striated microfilament bundles extended from subapical zone to the central part of the cell. The bundle consisted of longitudinal fibers and transverse striations; the latter having a periodicity of ca. 60 nm along the length of the fibers. The diameter of the bundle reaches 80 nm in maximum with 60-70 nm in average and the longest one sectioned was 4 μm in length. The discontinuity of the microfilament bundles with both ends tapered in the plane of section probably due to their undulated arrangement within the cytoplasm (Fig. 12). Woronin body plugged a septal pore of the apical cell of conidiophore immediately before conidium formation (Fig. 13). Woronin bodies were found on both sides of the septum and one on apical side plugged the pore with concomitant development of vacuole in the apical, conidiogenous cell.

In the apical cell of conidiophore which was induced dedifferentiation, concentric swirls of membranes (myelin figures) within the beginnings of a vacuolar system were found throughout the cell (Fig. 14). Mitochondria were constantly associated with the beginnings of a vacuolar system. A mitochondrion and a myelin figure within a vacuole were seen to be connected as indicated by an arrow in Fig. 15.

At the neck of an immature conidium, both of the inner and outer layers of cell
Fig. 8. Apical cell of conidiophore immediately after 2 hr blue light irradiation. The 2 hr blue light interruption was given after 6 hr darkness following conidiophore induction by 6 hr NUV irradiation. Note the apical dome without electron transparent structure and dispersion of small vacuoles (V) around the area of nuclei (N). There is no indication of vegetative regrowth at this moment. Scale=1 µm

Fig. 9. Apical portion of conidiophore showing the beginning of vegetative regrowth. Two hr darkness after 2 hr blue light interruption. Note the parabolic contour of the apex with thin and electron transparent cell wall. There are many apical vesicles (A) in the apical zone of cytoplasm. Several vacuoles with myelin figures (MF) are observed. Small vacuoles (V) without myelin figures are also found. M: Mitochondria. Scale=0.5 µm

wall of the conidium did not continue to those of the conidiogenous cell (Fig. 16).

Discussion

Light-microscopic observation revealed that the cell wall of the conidiophore apex pigmented heavily and a thick osmiophilic outer layer was found in the same portion by an electron microscopy at the stage immediately before initiation of conidium formation. The thick osmiophilic layer is a melanized cell wall as in *Alternaria brassicicola* (Schw.) Wiltshire and the pore for conidium formation is first produced presumably enzymically in this layer as suggested by Campbell (1969). A thickened portion of the layer forms an annulus around the pore (Fig. 6), and it may have a function to support and fix the new-born conidium to the apex of conidiophore.
Electron transparent structure (FTS) was specifically formed inside the apical dome of a fertile conidiophore which was kept under darkness after conidiophore induction (Fig. 1, 2). This structure did not form in a conidiophore which was induced to dedifferentiate into a vegetative hypha by blue light irradiation (Fig. 8, 9). By comparing apical structures of vegetative mycelium and germ tube, Bracker (1971) pointed out that washing and centrifuging of germinated spores before fixation caused disruption of apical vesicles and a divergence of the plasma membrane from its normal position against the cell wall. This fact indicates a possibility that ETS is an artifact of divergence of the plasma membrane at the apical dome of conidiophore. However, we concluded this was not the case based on the following reasons: 1) Under the same treatment, ETS was not formed in the conidiophores exposed to 2 hr blue light. 2) The treatment before fixation was just cutting out of conidiophores with an agar medium and samples were immediately brought into the fixative. So the time before the fixation was quite short and the treatment was more mild compared with the case for the germinating spores. 3) Apical vesicles were found not only in the apical zone of cytoplasm of the dedifferentiated conidiophores but also in the central cavity of ETS of the fertile conidiophores.

In the apical zone of conidiophores in *Stemphylium botryosum* Wallroth, Carroll (1972) found the speckled substance which was seen to be alike with ETS of *Alternaria solani* in this study. However, this substance has a smaller size than ETS and was not found at the beginning stage of conidium formation. Speckled substances were considered to be involved in cell wall synthesis and it seems to have completely different identity from ETS. In the same fungus, Carroll and Carroll (1971) observed fibrillar elements in vesicles around the pore of the conidiophore apex. A radial array of fibrils perpendicular to the plasma membrane formed the cytoplasmic neck in the conidiophore apex. The cytoplasmic neck thus formed is seen to be alike with the cytoplasm surrounded by ETS suggesting a similar
nature of ETS in *A. solani* and the vesicles with fibrils in *S. botryosum*.

Further more Carroll and Carroll (1971) showed that a zone of plasma membrane convolution appeared first in the middle cells of conidiophore, then at the apex of the conidiophore. One transverse section of conidiophore apex in *S. botryosum* showed myelin like swirls of plasma membrane surrounding the nucleus. Although we did not observe a transverse section of conidiophore apex in *A. solani*, myelin figures associated with ETS in the longitudinal section might have the same conformation with that of *S. botryosum* in the transverse section. In spite of the difference in the type of conidiogenesis, these two fungi may have the same cytoplasmic structures pertaining to conidium formation. Although constant association of myelin figures with ETS suggests that ETS originates from myelin figures, there is no evidence on

Fig. 11. Striated microfilament bundle (SMB) in the apical cell of conidiophore which was induced to initiate vegetative regrowth by blue light interruption. Four hr darkness after 2 hr blue light interruption. Striated microfilament bundle extends from subapical zone to the central part of the cell. Note small vacuoles (V) evenly distributed along the length of the apical cell. Scale=1 μm

Fig. 12. Striated microfilament bundle (SMB) with both ends tapered in the plane of section. The diameter of the bundle is 60-70 nm in average and the longest one was 4 μm in length. Periodicity of the striation is 60 nm. Scale=0.1 μm

Fig. 14. Small vacuoles (V) with or without myelin figures (MF) in an apical cell of conidiophore which was induced to initiate vegetative regrowth by blue light irradiation. Note an even distribution of small vacuoles along the entire length of cell. N : Nucleus. Scale=2 μm

Fig. 15. Myelin figures (MF) within the beginnings of vacuoles (V) and associating mitochondria (M). Scale=0.2 μm
the matter and the developmental process of ETS is yet to be explored.

As a role of ETS in conidium formation we speculate that ETS restricts the cytoplasm to contact with cell wall only in a small portion of the conidiophore apex, thus leading to formation of a pore or channel by dissolving both the inner and outer layers of cell wall. The pore surrounded by thickened cell wall, or an annulus restricts growing surface to a small portion of plasma membrane and this might bring the change in the type of apical growth from a cylindrical type

Fig. 13. Woronin body (WB) plugging a septal pore (SP) of apical cell from distal side of conidiophore. After 8 hr darkness following conidiophore induction by 6 hr NUV irradiation. S: Septum, W: Cell wall. Scale=0.5 μm
to a spherical type to form the round conidium initial covered only with the plasma membrane (Fig. 4, 5, 6).

In the conidiophore which was exposed to blue light, on the other hand, ETS did not form and the whole surface of apical dome expanded with a steep descending gradient in the rate of cell wall expansion within the dome as being presumed by Green (1969) resulting in dedifferentiation of conidiophore to form a vegetative hypha with a smaller diameter than the conidiophore (Fig. 9, 10).

In addition to the inhibition of ETS formation, blue light inhibited the vacuoles to expand and coalesce into a large vacuolar system, thus resulted in a relatively homogeneous distribution of small vacuoles throughout an apical cell of the conidiophore (Fig. 14). The turgor pressure provides the driving force that extends the apical wall in a vegetative hypha (Bartnicki-Garcia, 1973). Development of vacuoles along the length of mycelium may increase turgor pressure (Fig. 3) and it is believed that vacuoles push cytoplasm to the direction of the apex (Tanaka, 1986). It seems reasonable to believe that the formation of porogenous conidia as in the case of A. solani needs higher turgor pressure, since the vast cytoplasm has to pass through a small pore to form a conidium. A developed vacuole might provide the high turgor pressure required to form the conidium. Concerted plugging of a septal pore by Woronin bodies in the apical cell is thought to be necessary to make the high turgor pressure effective to push out the cytoplasm through a small pore at the apex to form a conidium.

Through the inhibition of development of a large vacuolar system, blue light brings the conidiophore into a juvenile mycelium with many small vacuoles containing myelin figures (Yanagita, 1981). Carroll and Carroll (1973) stated that the appearance of myelinoid bodies was connected with cell senescence in the conidiogenous cell of Stemphylium botryosum, and the production of myelinoid bodies was initiated by fusion of small cytoplasmic vesicles with the mitochondrion. Our observation on the appearance of the small vacuole containing myelin figures (Fig. 14) is different from Stemphylium. Instead of the senescent hyphae, they appeared in the juvenile hyphae which were induced to revert from conidiophores by blue light irradiation. Formation of the myelin figures may take place through the engulfment of mitochondria by small vacuoles (Fig. 15). Thus we conclude that the blue light induces dedifferentiation of the conidiophore by inhibiting the development of both the electron transparent
structure and the large vacuolar system in the apical cell of the conidiophore.

Filament bundles were reported to be representative of subcellular differentiation accompanying induction of the sexual phase in *Ascobolus stercorarius* (Bull.) Schroet. (Anderson and Zachariah, 1974). Also a non-membrane-bounded fascicle of microfibrils was regularly found within the fertile hyphae, or conidiophores in *Drechlera sorokiniana* (Sacc.) Subram. et Jain (Cole, 1972). Striated microfilament bundles were reported in a basidium of *Russula* species (Gull, 1975) and in conidiogenous cells of *Pleiochaeta setosa* (Kirchn.) Hughes (Harvey, 1974). However, the structure of striated microfilament bundles of *A. solani* was different from those reported for *A. stercorarius*, *D. sorokiniana* and *Russula* species. Striated fibers with a 60-70 nm periobility and a diameter of up to 110 nm reported from the mycelium, conidiophores, immature conidia and germ tube of *P. setosa* (Harvey, 1974) have the structural similarities with the striated microfilament bundles of *A. solani* reported here. Although Tanaka (1985) pointed out that these microfibrillar structures are closely associated with the process of both sexual and asexual reproductions, the striated microfilament bundles were not found in the fertile conidiophores in *A. solani*, and they seem to be connected with dedifferentiation, or vegetative reversion of conidiophores.

Since Hughes's proposal (Hughes, 1953) to separate Hyphomycetes into eight Sections based on characters of conidiophore and conidium development, a question has been introduced as to whether porogenous conidia actually arise as protrusions of the protoplast through pores dissolved in the conidiophore wall and are, therefore, fundamentally different from blastogenous conidia, or whether the 'pores' are merely 'thin areas' in the conidiophore wall that bulge outward to form the conidium initial in much the same fashion as in the formation of blastogenous conidia (Luttrell, 1963). Carroll and Carroll (1971) provided the evidence to this question that at least in *S. botryosum* the primary walls of conidiophores and conidia are continuous until the time of conidium secession. This result contradicted Hughes's initial diagnosis (Hughes, 1953) that although poroconidia might be formed by a budding process, a sharp discontinuity is maintained between the conidium and conidiophore walls. Our results differ from *S. botryosum*. At the pore of the conidiophore of *A. solani* the outer and inner layers of cell wall dissolved completely and the cytoplasm covered only with plasma membrane protruded through the pore to form conidium initial (Fig. 5). Later on the cell wall was formed around the conidium, but discontinuity between the walls of a conidiogenous cell and a conidium maintained through all stages of conidium development (Fig. 16) in accordance with Hughes's initial diagnosis of the porospore.

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摘 要

「アルナリアサギノキリ」 Alternaria solani の分生子発の微細構造を電顕観察し、分生子胞子形成の先端形成の先端子胞と青色光の照射によって脱分化する先端子胞の細胞内構造の違いを明らかにしようとした。分生子胞子形成する前生子胞子先端部には電子透過構造（Electron transparent structure, ETS）が形成され、先端子胞子と同様の細胞質が小さな部分で細胞壁と透明するよう制限を加えている。電子透過構造の発達に合わせて、先端子胞の先端子胞は大量の液泡が発達する。発達した液泡は、分生子胞子先端に形成された小さな穴から細胞壁を被った細胞質を押し出し、分生子胞子形成に対する必要とされる膨張をつくり出すと考えられる。先端子胞子は青色光の照射を受けたことによって、電子透過構造が増大し、液胞の発達を共に阻害される。分生子胞子の形成ではなく発酵菌系の形成へと脱分化する。