Developmental Anatomy of Experimentally Induced Ascocarps in *Gelasinospora reticulispora*

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In a sordariaceous fungus, *Gelasinospora reticulispora*, ascocarps induced by white light irradiation after a period of dark incubation or by aging of the culture were typically ostiolate perithecia. But those triggered by the arrival of the hyphal tip at the distal end of the growth tube were nearly always devoid of an ostiole, so that spores were discharged in a cleistothecial manner. The asci developed at a similar rate irrespective of the methods of induction. In all the cases ascocarps were formed at various depths of the agar medium, and the orientation of asci was totally at random although it always coincided with the orientation of the ostiole if present.

Ascocarp development has hitherto been studied in many fungi (Alexopoulos, 1962; Turian, 1966) including *Gelasinospora* (Meyer, 1957; Ellis, 1960), but little has so far been known about the time-course for ascocarp development. Although there have been a few descriptions of ascocarp formation with time scales (Ellis, 1960), none of them have started at the time of initiation but always arbitrarily at some point of development. Thus, it would be essential for time-course studies to have a system in which the time of ascocarp induction is experimentally controlled.

Recently, Inoue and Furuya (1970) reported that ascocarps were synchronously induced in a homothallic fungus, *Gelasinospora reticulispora* (Sordariaceae) when the tip of the hyphae incubated in a growth tube reached the distal end of the tube, or when a white light exposure was given after a period of dark incubation while the hyphal tip was growing. They reported also that ascocarps were gradually produced due to aging of the culture. Therefore the time of ascocarp initiation can be determined by using a growth tube of an appropriate length to terminate the hyphal tip growth at a desirable time, or by treating with light after incubation in the dark. In the former the ascocarp formation began at the youngest portion of the culture and proceeded basipetally toward the inoculated end, while in the latter, it began at the basal end of culture and extended acropetally.

The present investigation was made to compare the time-course and morphological patterns of ascocarp formation induced by these triggers.

Materials and Methods

A culture of *Gelasinospora reticulispora* designated as IFO 8367 was obtained from the Institute of Fermentation, Osaka, and the strain used in this study was derived from

a single ascospore. Culture procedures and light sources were previously described (Inoue and Furuya, 1970). Samples were obtained as an agar block (about 1 cm long $\times$ 1 cm in width $\times$ 3 mm in depth) from the region of the growth tube where the earliest ascocarps had appeared. They were then fixed in formalin propionic alcohol (Sass, 1958). After dehydration and infiltration through an ethanol-normal butanol series, samples were embedded in paraffin. Sections were cut transversely with regard to the growth tube by means of a rotary microtome to the thickness of 10 μ and stained with safranin O and fast green FCF.

It was certified in a preliminary test that the locality, form or orientation of ascocarps did not change markedly in the procedures of anatomical preparation described above.

All of the photographs in the text are arranged so that the direction from the top to the bottom of the photographs represent that of gravity during cultivation.

Results

Ascocarps induced in total darkness by the arrival of the hyphal tip at the distal end of medium: In total darkness the hyphal tip reached the distal end of 7-cm-long growth tube within a range of 51–63 hr of incubation, and ascocarps became visible about 35 hr thereafter; i.e. 84–96 hr after the inoculation.

During the first 21–33 hr after the arrival of the hyphal tip at the distal end of the medium, no sign of perithecial formation was noted except occasional irregular-shaped tangles of hyphae about 20–40 μ in diameter on the surface and at deeper places of the medium (Fig. 1). These structures appeared to be later stages of protoperithecial initials described by Ellis (1960), although earlier stages were not observed in the present work.

With samples fixed at 108 hr after inoculation, primordial ascocarps were globose in shape and 40–220 μ in diameter, and were found at various depths of the agar medium including both the surface and the bottom (Fig. 2). No sign of ascus differentiation was perceived, although the peridium was well distinguished as tight pseudoparenchyma of little intercellular space from centrum, loose pseudoparenchyma, enveloped within. Peridia were 3–9 cells in thickness and the outermost cells were more or less thick-walled.

By the 131st hr of the culture, the globose organ developed both in size and in structure, its diameter reaching 60–290 μ . At this time many primordial asci were recognized near the margin of the loose pseudoparenchyma as a cluster of short club-shaped cells with dense cytoplasm (Fig. 3). Occurrence of such clusters was not restricted to the lower side of the centrum but the orientation differed at random from ascocarp to ascocarp. The peridium was 5 to 10 cells in thickness, and the outermost 1–4 layers were composed of thick-walled cells.

At 155 hr after inoculation, neither the size nor the shape of the peridium showed any remarkable change if compared with those at a day-earlier stage, while the primordial asci had become larger in size at the expense of the surrounding cells which

Figs. 1-4. Successive stages in ascocarp development in 7-cm-long growth tubes kept in the dark. Figs. 1, 2, 3 and 4: 84, 108, 131 and 155 hr, respectively, after inoculation. In preparing the text figures the photographs were always arranged so that the top and bottom represent the direction of gravity. To show ascocarps which have some structural polarities, photographs of longitudinally sectioned ascocarps were selected.

shrivelled markedly (Fig. 4). Some of those primordial asci had more than two nuclei, but no mitotic figure was discovered.

At 179 hr after inoculation, most space inside the peridium was filled up with asci containing eight spores and no paraphysis was observed with certainty (Fig. 5). The ascospores were not always arrayed in a single row but were often more or less irregularly packed in an oval or broadly ellipsoidal ascus.

The wall of the ascospores had become somewhat darker in color and showed a reticulate sculpture in 194-hr-old cultures, when most of asci had a slender cylindrical shape and uniseriately arrayed spores inside (Fig. 6). The darkening of the ascospore

Figs. 5-8. Successive stages in ascocarp development in 7-cm-long growth tubes kept in the dark. Figs. 5, 6, 7 and 8: 179, 194, 243 and 360 hr, respectively, after inoculation.

wall was not due to the stains, since unstained preparations also showed such a tendency.

In 243-hr-old cultures, asci contained darker and more deeply sculptured spores (Fig. 7). The peridium of most ascocarps was slightly deformed in an irregular manner. The deformation was not attributable to an artifact because the slides were made in the same way as for other samples in which more evenly spherical peridia were observed.

Ascospores in 360-hr-old cultures had become so dark that the wall sculpture was hardly discernible under a microscope (Fig. 8). From some of the ascocarps, spores had been discharged through one or more fissures on the peridium. According to the common terminology, the ascocarps should be called cleistothecia rather than perithecia. Ascocarps had a somewhat irregularly deformed peridium when all the spores

Table 1. Frequency of types of ascocarps induced in the dark by arrival of hyphal tips at the distal ends of 7-cm-long growth tubes at the 53rd hr after inoculation. Data were obtained from observation of serial sections through apical 1 cm of the culture.

Types of ascocarps	Number of ascocarps (%)	
	360 hr after inoculation	458 hr after inoculation
Cleistothecia	3 (6)	5 (31)
Ostiolate perithecia	1 (2)	0 (0)
Immature ascocarps	44 (92)	11 (69)
Total ascocarps observed	48 (100)	16 (100)

were kept inside, whereas, after some of the spores had been released, they tended to have more or less evenly spherical walls except for the fissures. Among the 48 ascocarps observed, only one was provided with an ostiole. Judged by the darkness in color and sculpture on the wall, the spores in the ostiolate perithecium were as mature as those in most of the other ascocarps.

No remarkable change of ascocarp structure was noted with 458-hr-old cultures except for an increase in the rate of ascocarps from which the spores had been discharged in a cleistothecial manner (Table 1).

Ascocarps induced under continuous white light by the arrival of the hyphal tip at the distal end of medium: The rate of ascocarp development in the light was similar to that of development in the dark. The hyphal tip reached the distal end of the medium at 50–65th hr after inoculation, and globose organs began to be visible about 25 hr thereafter, i.e. 72–84 hr after inoculation. Ascocarps of 130-hr-old cultures in the light (Fig. 9) seemed comparable in size, shape, structure and distribution with those in the 155-hr-old dark culture. At the 360th hr, no distinction was found between the dark and light cultures. The position of fissure(s) on the peridium and the orientation of asci inside were totally independent of each other. Among 117 ascocarps observed, only six had a normal ostiole lined with periphyses. One of them had lightly colored, thin-walled ascospores (Fig. 10) while the rest of them and most of the ascocarps without ostioles had very darkly colored, thick-walled ascospores inside and/or outside the peridium (Figs. 11 and 12).

Ascocarps photoinduced after dark incubation: Cultures were placed in total darkness for the first 53 hr, and then were exposed to white light for the rest of the culture period so that the initiation of the ascocarp was triggered approximately at the same age of the culture as in cases described in the previous chapters. When the ascocarps were thus induced, an ostiole, a typical characteristic of a perithecium, was formed in almost all of them. The orientation of ostioles was always identical to that of asci, but the asci directed quite at random. For observation of ascocarps in younger stages, 33-cm-long growth tubes were used which allowed hyphal tip growth up to about 200 hr. Sixty-cm-long growth tubes were provided for longer periods of tip growth.

Primordial ascocarps became visible 83–107 hr after inoculation, i.e. 30–54 hr after

Figs. 9-12. Ascocarps in 7-cm-long growth tubes kept under continuous light. Fig. 9: an immature ascocarp containing many primordial asci as seen in a section through 130-hr-old culture. Fig. 10: an ostiolate perithecium containing lightly colored, thin-walled ascospores as seen in a section through 360-hr-old culture. Figs. 11-12: two of the serial sections through a cleistothecium in 360-hr-old culture showing spore discharge through three fissures.

exposure to light. At the 108th and 130th hr after inoculation, ascocarps quite similar in morphology to those shown in Figs. 2 and 3, respectively, were observed. But a few ascocarps were pyriform and, in spite of the immaturity of asci, had an easily recognizable ostiole lined with periphyses. Some ascocarps were ovoid in shape having an ostiolar protuberance, i.e. a protuberance suggestive of a future ostiole. Globose ascocarps without any sign of ostiole formation were fewer than ovoid and pyriform ones in 155-hr-old cultures and were rare in 194-hr-old cultures. The asci in 155- and 194-hr-old cultures were in slightly progressed stages compared with those in dark-grown cultures of the corresponding ages in which the ascocarp formation was induced by the arrival of the hyphal tip at the distal end of medium (Figs. 4 and 6). Eight thin-walled ascospores were present in each ascus at the 155th hr (Fig. 13), and wall reticulation was clearly seen at the 194th hr (Fig. 14).

Ascocarps induced by aging in the dark: To allow hyphal tip growth during the periods of experiments, 33-cm-long growth tubes and 60-cm-long, U-shaped ones were used. In dark-grown cultures perithecia became discernible near the inoculum about

Figs. 13-16. Stages in development of perithecia induced by irradiation following a dark period of 53 hr (Figs. 13 and 14), and by aging in the dark (Figs. 15 and 16). Figs. 13, 14, 15 and 16: 155, 194, 194 and 233 hr, respectively after inoculation.

122-145 hr after inoculation. At the 147th hr primordial ascocarps were found at varying depths, and closely resembled in morphology those observed in 108-hr-old dark cultures in 7-cm-long growth tubes, of which an example is shown in Fig. 2. No sign of ostiole formation was noted at this time. The asci observed at the 194th hr (Fig. 15) reached to the stage of the 155-hr-old dark culture in 7-cm-long tubes. Some of the ascocarps, however, had an ostiole or an ostiolar protuberance. The orientation of the ostiole or ostiolar protuberance always coincided with that of the asci inside.

The asci and ascospores at the 233rd (Fig. 16) and the 281st hr were very similar in morphology to those observed at the 194th (Fig. 6) and the 243rd (Fig. 7) hr, respectively, in ascocarps induced in the dark by the arrival of the hyphal tip at the distal end of medium. By the 233rd hr ascocarps without any sign of ostiole formation were

Fig. 17. Diagram showing approximate time-course of ascocarp development triggered by varied conditions. †: arrival of the hyphal tips at the distal end of medium; *: start of white light irradiation; ?: estimated time of ascocarp induction; ↑: appearance of the earliest ascocarps. Solid and open bars indicate the periods in which the material was kept in total darkness and under continuous white light, respectively.

very rare. Ascocarps formed under this condition, therefore, should be called perithecia.

Ascocarps induced by aging under continuous white light: Although it was reported that no visible ascocarp occurred in cultures placed under continuous white light for 7 days (Inoue and Furuya, 1970), ascocarps were formed in the cultures even under continuous white light when incubated for a longer period of time. Ascocarp development took place very similarly to that just described, except for about 2 days of retardation, counted from the inoculation. Ascocarps observed at the 194th hr and at the 328th hr looked like those found at the 108th (Fig. 2) and 243rd (Fig. 7) hr, respectively, in the dark cultures in which the ascocarp formation was induced by the arrival of the hyphal tip at the distal end of medium. Ostiolate perithecia were observed in the later stage.

Discussion

Results of time-course studies on ascocarp formation in the present work are summarized in Fig. 17. Development of asci showed no marked difference either in speed or in pattern irrespective of light conditions or tube lengths. In all the cases examined when the timing of ascocarp induction was known, the time length required for asci in a certain developmental stage to be formed was approximately the same.

Assuming that this is also true with perithecial development caused by aging, the timing of induction by aging can be estimated as about 100 hr and 150 hr after inoculation in the dark and under continuous light, respectively. In contrast to timing of ascus formation, the timing of ostiole formation varied widely even in the same culture; in some perithecia it occurred before the appearance of ascus primordia, but in others it was after the establishment of eight spores in each ascus.

Developmental processes of the peridium, especially in later stages, varied markedly with experimental conditions. Ascocarps induced by the arrival of hyphal tips at the distal end of the medium were nearly always cleistothecial in the manner of spore discharge while those induced by other triggers were perithecial, i.e., provided with an ostiole.

It is suggested that the two phenomena, peridium development and ascus formation, might be controlled by different factors. Such a possibility is also suggested by Olive and Fantini (1961) who observed abundant "empty perithecia", perithecia of mature size but devoid of asci, at the line of contact between certain mating types of *Gelasinospora autosteiira* and *Sordaria brevicollis*.

It is noteworthy that a single clone of a sordariaceous species produces cleistothecia under some environmental conditions and produces perithecia under others. This leads us to a concept that under some appropriate conditions perithecia can be formed in a species which has hitherto been considered to produce cleistothecia only, and *vice versa*. In this respect, perithecial or cleistothecial phenotype seems to be of little value at taxonomic levels higher than the genus (Cain, 1961). Furthermore, it can be said that in the case of the genus *Gelasinospora* ascocarp phenotype is of no taxonomic value at any rank. Maniotis's study on a cleistothecial mutant of *Gelasinospora calospora* (1965) strongly supports this opinion.

A few ostiolate perithecia were discovered in aged cultures in 7-cm-long growth tubes. One of such exceptional perithecia contained lightly colored, thin-walled ascospores and was supposed to have been induced later than other ascocarps in the same tube owing to aging of the culture.

Orientation of asci differed at random with ascocarps although it always coincided with that of the ostiole (if present) of the same ascocarp. Therefore it is ascertained that the orientation is determined by some other factor(s) than gravity. Independence of the orientation from phototropism, however, is not ensured, because inflexion and reflexion by glassware vessels and agar medium are too complex to be ignored. In cleistothecia the positions of rupture and orientation of asci were independent of each other. Some cleistothecia had two or three ruptures toward varied directions. The positions of the ruptures seem to be determined accidentally by the mechanical natures of the peridial wall, contained asci and surrounding agar.

Another noteworthy result of observation is the depth in the agar medium where ascocarps are formed. Alexopoulos (1962) adopted distribution of ascocarps as a key character to distinguish Sordariaceae from Phyllachoraceae in stating that they are superficial in the former and immersed in the substratum in the latter. Under every

experimental condition of the present study, however, ascocarps were formed at varied depths; that is, some were on the surface, but others were in the middle or near the bottom of the agar medium. Therefore, superficial or immersed distribution of ascocarps is of no taxonomic value for material cultured under certain experimental conditions.

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