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Effects of high-temperature treatment on two essential light processes and an intervening dark process in photoinduced pileus primordium formation of a basidiomycete, *Favolus arcularius*

Yutaka Kitamoto[1], Katsuji Akita[1] and Takao Horikoshi[2]

[1] Department of Bioscience and Biotechnology, Faculty of Agriculture, Tottori University, 4–101, Minami, Koyama-cho, Tottori 680–0945, Japan

[2] Department of Environmental Sciences, Faculty of Integrated Arts and Sciences, Hiroshima University, Kagamiyama, Higashihiroshima 739–8521, Japan

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The photoinduced formation of pileus primordia in *Favolus arcularius* involves two essential light processes and an inserting dark process. The nature of these elementary processes in epileate stipes was examined by the use of high temperature under a 1-h light-7-h dark-continuous light regimen. Epileate stipes were exposed to a temperature treatment of 37°C for 15 min after the beginning of pileus primordium formation, which disrupted the photomorphogenetic progress without any after-effects. When high temperature was applied in the first light process or the early phase of the dark process, it completely voided the established career. A temperature-sensitive key dark reaction may have occurred in the period of 0–2 h. When high temperature was applied during the late phase of the dark period, it caused only a delay in pileus primordium formation, suggesting that the high temperature might only retard the progression of the dark morphogenetic reaction in this period. In addition, the early phase of the second light process was effectively disrupted by the use of high temperature, but sensitivity to high temperature gradually decreased with the progress of pileus differentiation.

Key Words——basidiomycete; *Favolus arcularius*; high-temperature effect; photomorphogenesis; pileus formation.

Favolus arcularius (Fr.) Ames is a polypore mushroom that needs light for two stages of fruit-body development: formation of the fruit-body primordium, and formation of the pileus primordium (Kitamoto et al., 1968). The action spectrum for primordium formation lies in the region between near UV and blue light and coincides substantially with that for pileus primordium formation (Kitamoto et al., 1972, 1974), indicating the involvement of a common photoreceptor system in these photomorphogenetic responses.

Horikoshi et al. (1974) found that the photoinduction of pileus primordium formation in *F. arcularius* strain 69A (ATCC 24460) involved two light-requiring processes and an intervening light-independent (dark) process. The first light process took place during the first hour, and the second from 8 to 24 h. The photoresponse in the first light process seemed to have a threshold intensity value, being saturated by only 5 lx of light. In contrast, the second light process was accelerated by an increase in light intensity up to about 150 lx. In addition, photoinduction of primordium formation also involved a two-step light process and an intervening dark process, each with apparently similar characteristics to the corresponding three elementary processes in pileus primordium formation (Kitamoto et al., 1986). To date, however, the detailed nature of these light and dark processes in the two different developmental stages of fruit-body formation in *F. arcularius* is unclear.

In analyzing the physiological characteristics of the elementary processes involved in photomorphogenetic events in basidiomycetous fungi, it is useful to be able to the control each process by adopting external physical treatments that do not cause after-effects. In the photomorphogenesis of basidiomycetes, however, limited information is available on the disruptive effects of interpolating a high-temperature period at different stages in the development of fruit-bodies. Perkins and Gordon (1969) indicated that the fruiting of *Schizophyllum commune* Fr.: Fr. could be disrupted by exposure for 1 h or longer to 50°C immediately after photoinduction; vegetative growth, however, was not apparently injured by the use of heat. In the maturation of fruit-bodies in *Coprinus lagopus* (Fr.: Fr.) Fr. (*Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray), Lu (1972) showed that this fungus could enter and complete meiosis at 25°C under a continuous light regimen, but was unable to do so at 35°C. Consequently, he determined the time programmed for the initiation of meiosis, using high-temperature arrest with a combination of inductive light. In this study, characteristics of the two light-requiring process-

es and the intervening dark process of photoinduction in epileate stipes were examined by a high-temperature method in cultures under a light and dark regimen.

Materials and Methods

Test organism A dikaryotic strain (ATCC24461/69B) of *F. arcularius* was used. This strain is more sensitive to light than strain 69A/ATCC 24460, which we used in previous studies (cf. Kitamoto et al., 1968, 1986; Horikoshi et al., 1974). It completed the stages of primordium formation and pileus primordium formation within shorter periods than did the latter strain.

Culturing of the epileate stipes for synchronization of pileus primordium formation To induce pileus primordium formation synchronously under complex programs of light and temperature, dark-grown epileate stipes were used (cf. Horikoshi et al., 1974). A semisynthetic liquid medium was used for the test cultures (Kitamoto et al., 1972). It was composed of: maltose, 20 g; peptone, 1.0 g; KH_2PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.3 mg; $FeSO_4 \cdot 7H_2O$, 0.15 mg; $CuSO_4 \cdot 5H_2O$, 0.1 mg; $MnSO_4 \cdot 5H_2O$, 0.1 mg; $(NH_4)_6Mo_7O_{24} \cdot H_2O$, 0.02 mg; thiamine hydrochloride, 0.5 mg, niacin, 0.1 mg; distilled water, 1 L; and pH was adjusted to 5.6 with 0.1 M NaOH. Portions of 8 ml of the medium in 50-ml Erlenmeyer flasks were autoclaved at 110°C for 10 min. The agar medium for the seed culture was prepared by adding 1.5% agar to the liquid medium, and 12-ml portions of the medium were poured aseptically into 9-mm plastic Petri dishes after autoclaving. Plates were inoculated with a small mycelium-agar fragment from the stock culture and incubated in the dark at 25°C for 1 wk. Mycelial colonies grown on the plates were dissected into mycelium-agar blocks of $3 \times 3 \times 3$ mm and inoculated into the liquid medium for the test cultures. The cultures were preincubated at 25 ± 1°C in the dark to allow mycelial growth for 6–7 d, then fruiting was induced by continuous light exposure at 500 ± 50 lx provided by daylight fluorescent tubes, and a number of fruit body primordia arose on the colony. When cultures with fruit body primordia were transferred into the dark and incubated for 2–3 d, most of the primordia developed into cream-colored epileate stipes. Exposing the epileate stipes to light led to synchronous photoinduction of pileus primordium formation.

High temperature In preliminary experiments to determine the time of each elementary process acting in the photoinduced formation of pileus primordia in strain ATCC 24461, the first light-dependent process was found to occur during the first hour, the dark process from 1 to 6 h, the second light-dependent process from 6 to 13 h, and pileus primordium formation was completed at about 15 h after the beginning of photoinduction. The dark reaction and the second light process were shorter by 2 h and 8–10 h, respectively, in strain ATCC 24461 and in strain ATCC 24460 (cf. Horikoshi et al., 1974).

High temperature was applied for different times in various periods during the course of pileus primordium formation in cultures incubated under a regimen of 1-h light (for the first light process) – 7-h dark – continuous light (for the second light process) (1L-7D-CL regimen). The effects of temperature on the test cultures were evaluated by scoring the time required for the pileus primordium formation with the epileate stipes after the start of the first light exposure, and comparing it with that in the control cultures exposed to high temperature. A supplementary evaluation was done by scoring the proportion of cultures forming pileus primordia within 16 h after the start of the light exposure in the total number of cultures exposed to the same temperature. High temperature was applied by placing the culture flasks in a water-bath set at various temperatures with a deviation of less than 1°C.

Results

Effects of temperature on pileus primordium formation To examine the effects of high temperature on pileus primordium formation, epileate stipes were incubated at temperatures between 23 and 40°C under continuous light at 500 lx until the formation of pileus primordia. The results are shown in Fig. 1.

When epileate stipes were incubated at 23°C in the light, pileus primordia were formed at about 14 h after the beginning of photoinduction. The pileus primordium formation was slightly accelerated by incubating the epileate stipes under light at elevated temperatures up to 30°C. Epileate stipes incubated at 35 and 37°C, however, did not produce pileus primordia. On the other hand, when epileate stipes that had been treated at 37°C for 2 d were transferred to 25°C in the light, they formed pileus primordia, and completed their development. These results suggested that the use of high temperature could void the essential photomorphogenetic reaction for pileus primordium formation, and that the application of heat had no after-effect on the cultures.

Negative effects of the use of high temperature on the elementary processes involved in photoinduced pileus primordium formation The negative effects of the use of high temperature for different times in photoinduced pileus primordium formation were examined under the 1L-7D-CL regimen, as follows.

(a) Exposure to high temperature for the period of the first light process Epileate stipes were incubated at various temperatures during the first one hour of the 1L-7D-CL regimen. The results are shown in Fig. 2.

An inhibitory effect was observed in the cultures exposed to temperatures over 25°C. Pileus primordium formation was delayed by 2 h as compared with the control in the cultures exposed to 30°C, and by about 8 h in cultures exposed to 37°C. Therefore, the 37°C treatment substantially disrupted the photomorphogenetic effects established by the 1L, and the use of CL from 8 h re-induced the light reaction of the first light process in the epileate stipes.

The effect of exposure time at 37°C was examined. Exposure for 15 min at 37°C caused a delay of about 6–7 h in pileus primordium formation, and prolonging the exposure for up to 1 h strengthened the inhibitory effect

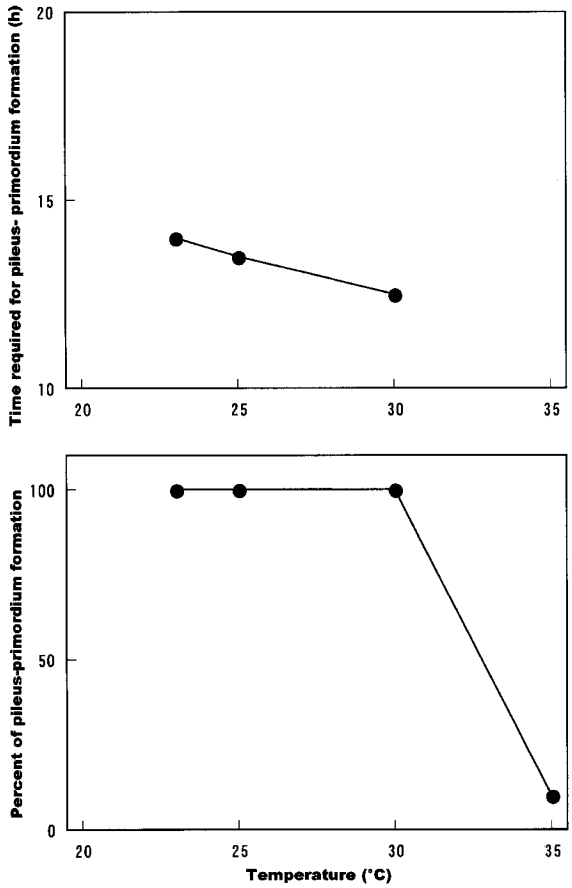

Fig. 1. Effect of different temperatures on the photoinduced formation of pileus primordia in *Favolus arcularius*.
Cultures with dark-grown epileate stipes were incubated under continuous light at different temperatures. Upper: time required for pileus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia within 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

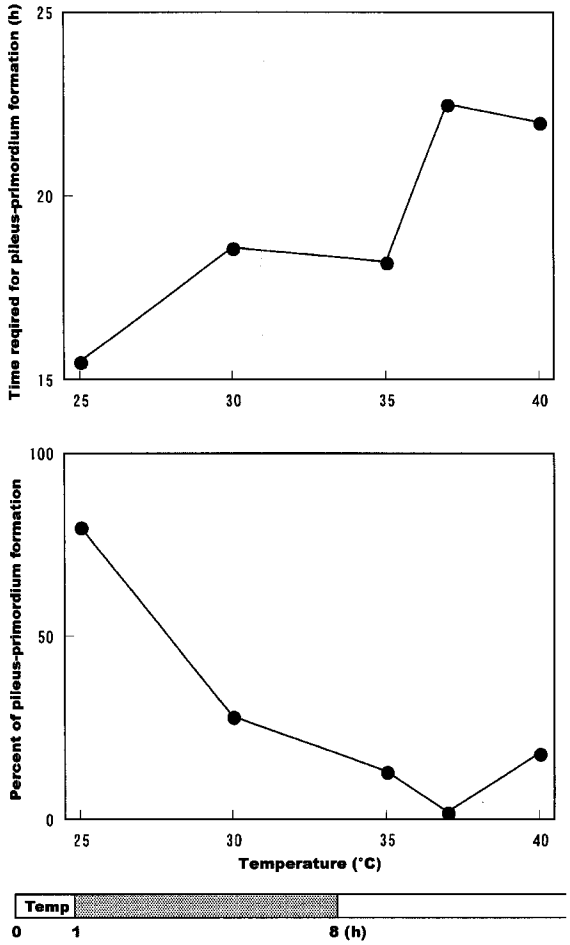

Fig. 2. Effect of different temperatures (**Temp** in the regimen) during the first light process of a 1L-7D-CL regimen on the photoinduced formation of pileus primordia in *Favolus arcularius*.
Cultures with dark-grown epileate stipes were exposed different temperatures for 1 h from the beginning of the first light exposure. Upper: the time required for pileus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia within 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

(Fig. 3).

(b) Exposure of high temperature during the dark process To examine the high-temperature effect on the dark reaction process, cultures with epileate stipes were exposed to a temperature of 25–40°C for 1 h beginning 3 h after the start of photoinduction under the 1L-7D-CL regimen. The results are shown in Fig. 4.

The formation of epileate stipes was delayed by exposure to temperatures over 30°C. However, the delay of about 4 h caused by exposure for 1 h at 37°C was significantly shorter than the delay of 8 h resulting from similar exposure during the preceding light process. Thus, the progress of pileus primordium formation in epileate stipes in the dark process could be retarded by high temperature incubation in this period, but sensitivity of epileate stipes to high temperature in this phase was lower than that in the initial 1L period.

(c) Exposure to high temperature during the period of the second light process To examine the effect of high temperature on the second light process, cultures with epileate stipes were treated at 25–40°C for 1 h beginning at 8 h under the 1L-7D-CL regimen. The results are shown in Fig. 5.

Temperatures below 30°C did not produce inhibitory effects, while treatment at 37°C delayed pileus primordium formation by 4 h. This indicates that exposure to high temperature at the beginning of the second light process did not completely disrupt the development begun in the two preceding photomorphogenetic processes, but it did retard it progress.

The time curve for the high-temperature sensitivity of the elementary processes in photoinduced pileus primordium formation The sensitivity to high temperature of the

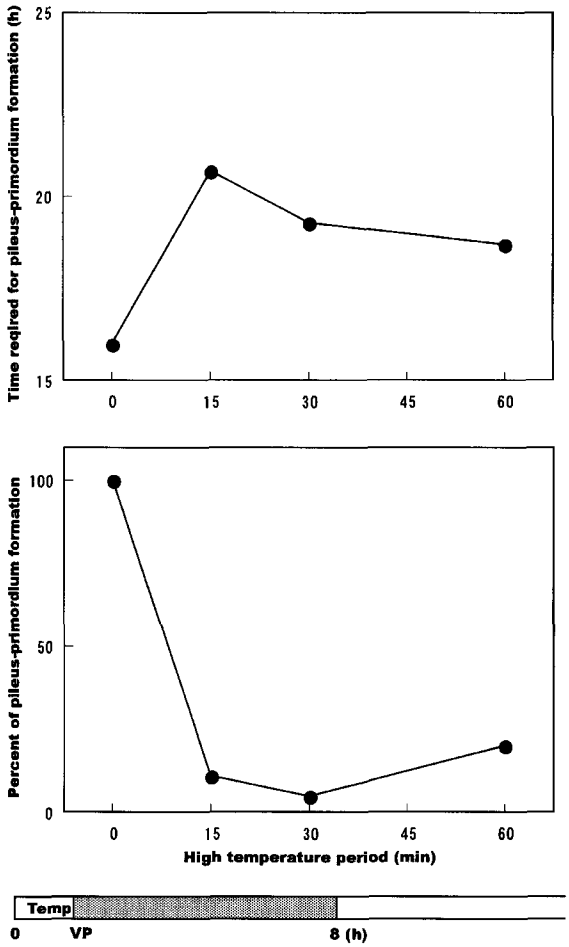

Fig. 3. Effect of various exposure periods to high temperature (**Temp** in the regimen) during the first light process of a 1L-7D-CL regimen on the photoinduced formation of pileus primordia in *Favolus arcularius*.
Cultures with dark-grown epileate stipes were exposed to 37°C for various periods (VP) from the beginning of the first light exposure. Upper: time required for pileus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia within 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

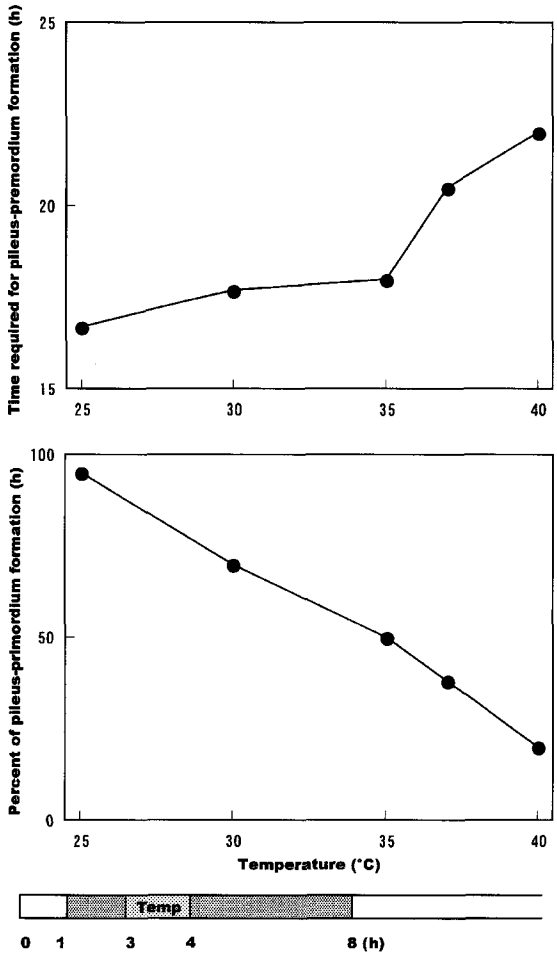

Fig. 4. Effect of high temperature exposure during the dark process (**Temp** in the regimen) of a 1L-7D-CL regimen on the photoinduced formation of pileus primordia in *Favolus arcularius*.
Cultures with dark-grown elipeate stipes were exposed to different temperatures for 1 h beginning 3 h after the start of the first light. Upper: time required for pileus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia with 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

elementary processes in pileus primordium formation was determined by exposure to epileate stipes at 37°C for 30 min under two different experimental light regimens. The results are shown in Figs. 6 and 7.

In the first experiment, cultures were exposed to high temperature at different times under continuous light. The control cultures (CL at 25°C) formed pileus primordia at 15.3 h after the start of photoinduction. When cultures were exposed to heat for 30 min at times up to 6 h, pileus primordium formation was delayed. A maximal delay of 2 h was caused by exposure at 4 h.

In the second experiment, cultures with epileate stipes were exposed to high temperature at different times under a 1L-7D-CL regimen. Exposure at 0.5 h after the start of light exposure delayed pileus primordium formation by 6 h as compared with the control, showing that the effect of light during the first light process was almost completely disrupted. Exposure to high temperature at 3-5th h elicited a delay of about 3–4 h in pileus primordium formation. And exposure at 10 h produced the maximum delay in pileus primordium formation, completely canceling the effects of the first light process.

Discussion

The natures of the elementary processes involved in the photoinduced pileus primordium formation of *F. arcularius*

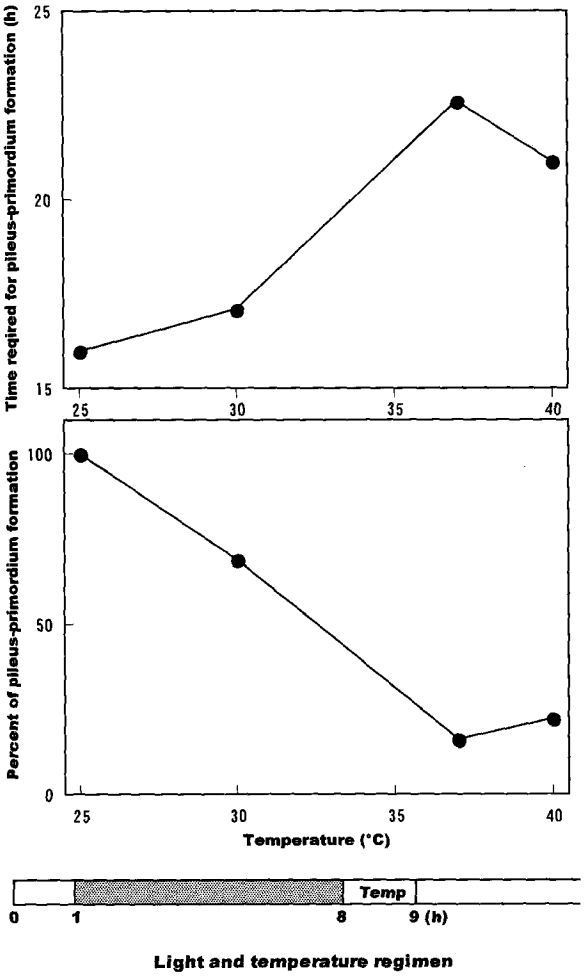

Fig. 5. Effect of high temperature exposure during the second light process (**Temp** in the regimen) of a 1L-7D-CL regimen on the photoinduced formation of pileus primordia in *Favolus arcularius*.
Cultures with dark-grown epileate stipes were exposed to different temperatures for 1 h from the beginning of the second light exposure. Upper: time required for pilus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia within 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

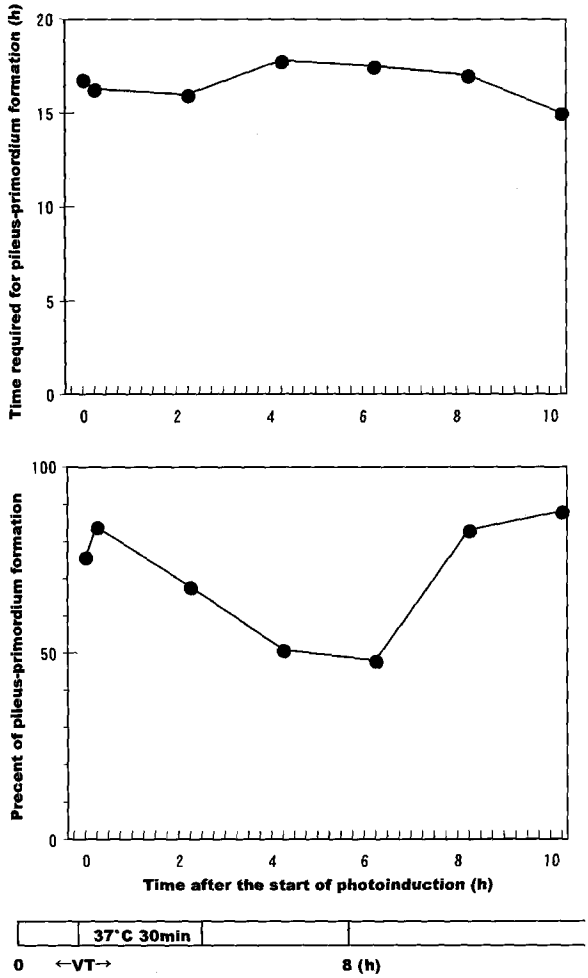

Fig. 6. Change in high-temperature sensitivity of the elementary processes in photoinduced pileus primordium formation during cultivation under continuous light of epileate stipes of *Favolus arcularius*.
Cultures with dark-grown epileate stipes were exposed to 37°C for 30 min at different times (VT) during the experiment. Upper: time required for pileus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia within 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

were examined by high temperatures. Exposure to 37°C for an appropriate time of cultures under-going pileus primordium formation completely disrupted the morphogenetic progress in this mushroom without any after-effect. A preceding report by Perkins and Gordon (1969) also described that the induction of fruit bodies by light was disrupted by post-induction heat-exposure of 50°C for 1 h or longer without any discernible effect of temperature, and that the periods following photoinduction were relatively more sensitive to heat in *S. commune*. Lu (1972) determined the programming by time for the initiation of meiosis in *C. lagopus* by using high temperature arrest method.

A previous study suggested that the photoreaction in the first light process act as a trigger to initiate pileus primordium formation (Horikoshi et al., 1974). In the present study using a 1L-7D-CL regimen, it was shown that the first light process and the early phase of the dark (independent to light-exposure) process were very sensitive to high temperatures, and high temperatures during the above periods completely disrupted the career of the first light process in the cultures, i.e., a key dark reaction for initiation of pileus primordium formation may occur during 1–2 h after the start of light exposure in the test strain. If high temperature treatment was applied under continuous light during period of 0–2.5 h, the delay of pileus primordium formation was only 1–2 h as compared with the control. For cultures grown under continuous

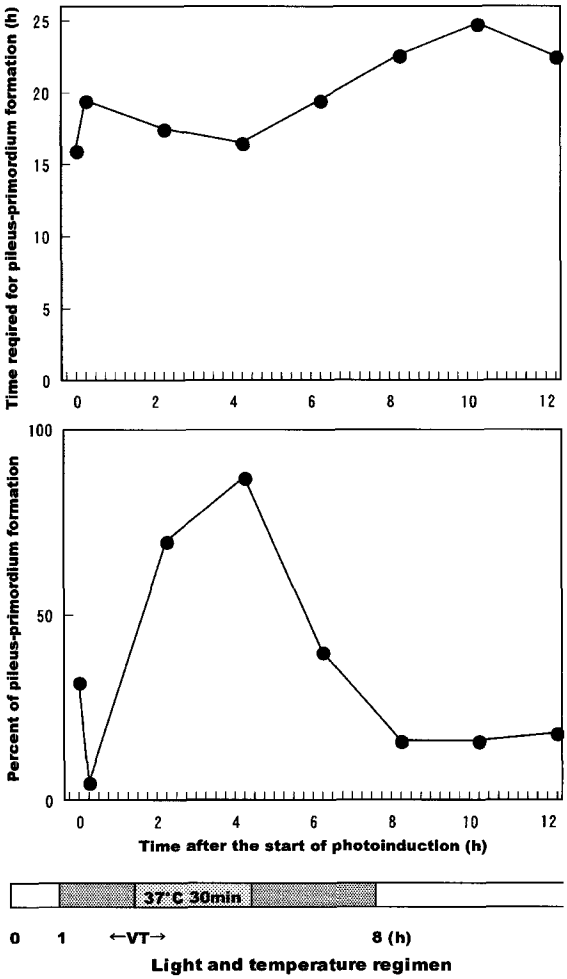

Fig. 7. Change in high-temperature sensitivity of the elementary processes in photoinduced pileus primordium formation during cultivation under a 1L-7D-CL regimen of epileate stipes of *Favolus arcularius*.
Cultures with dark-grown epieate stipes were exposed to 37°C for 30 min at different times (VT) during the experiment. Upper: time required for pileus primordium formation after the beginning of the light exposure. Lower: percentage of cultures forming pileus primordia within 16 h after the beginning of the light exposure. Twenty flasks were used for each replicate.

light environment, pileus development might be re-induced by the light on or just after the high temperatures.

Some features of the photoresponse in the second light process in pileus primordium formation were described in Introduction (cf. Horikoshi et al., 1974). Exposure to high temperature during the late phase of the dark period under a 1L-7D-CL regimen caused a delay in pileus primordium formation, but the career of the first light exposure in the cultures was not voided. It appears that the progression of the dark morphogenetic reaction that follows the key dark reaction was only retarded by the high temperature.

The early phase of the second light process was extremely sensitive to high temperature, but the sensitivity was gradually decreased with the progression of the second light process. When a high temperature was applied at about 10 h after the beginning of the first light process under a 1L-7D-CL regimen, the photomorphogenetic effects of the first light exposure were completely voided because the formation of pileus primordium was delayed by about 8 h.

Little is known on the molecular and biochemical backgrounds of the high temperature on photomorphogenesis in basidiomycetes. Horikoshi (1975) observed the photoinduced activation of RNA synthesis in the early phase of the dark process in the peripheral region of the apical 1 mm of the developing epileate stipes in *F. arcularius*. The biochemical event(s) following the photochemical reaction might be responsible for the sensitivity to high temperature. With the beginning of the second light process, abundant formation of septa and hyphal branching, and change in the orientation of hyphal growth to the horizontal direction in the apical region of stipe of *F. arcularius* were observed (Fukutomi et al., 1982). With this histological changes, many glycogen particles were accumulated in the cytoplasm along the cell walls, and the electron density of the multitubular structure increased in the photoinduced epileate stipe (Fukutomi et al., 1982). Once these histological and cytological features are established, their response to high temperature appear to become gradually less sensitive with time up to the formation of pileus primordium on the top of the stipe.

Literature cited

Fukutomi, M., Horikoshi, T., Akai, S. and Kitamoto, Y. 1982. Ultra structural aspects of photoinduced pileus formation in *Favolus arcularius*. Trans. Mycol. Soc. Japan **23**: 1–12.

Horikoshi, T. 1975. Studies on the photoinduced pileus formation of a basidiomycete, *Favolus arcularius*. PhD thesis, Kyoto University, Kyoto. (In Japanese.)

Horikoshi, T., Kitamoto, Y. and Kasai, Z. 1974. Effect of light on the initiation of pileus formation in a basidiomycete, *Favolus arcularius*. Plant & Cell Physiol. **15**: 903–911.

Kitamoto, Y., Horikoshi, T. and Suzuki, A. 1974. An action spectrum for photoinduction of pileus formation in a basidiomycete, *Favolus arcularius*. Planta **119**: 81–84.

Kitamoto, Y., Suzuki, A. and Furukawa, S. 1972. An action spectrum for light-induced primordium formation in a basidiomycete, *Favolus arcularius* (Fr.) Ames. Plant Physiol. **49**: 338–340.

Kitamoto, Y., Suzuki, A., Matsumoto, T. and Ichikawa, Y. 1986. The role of light on fruit-body initiation in *Favolus arcularius*. Trans. Mycol. Soc. Japan **27**: 451–461.

Kitamoto, Y., Takahashi, M. and Kasai, Z. 1968. Light-induced formation of fruit-bodies in a basidiomycete, *Favolus arcularius* (Fr.) Ames. Plant Cell Physiol. **9**: 797–805.

Lu, B. C. 1972. Dark dependence of meiosis at elevated temperatures in the Basidiomycete *Coprinus lagopus*. J. Bacteriol. **111**: 833–834.

Perkins, J. H. and Gordon, S. A. 1969. Morphogenesis in *Schizophyllum commune*. I. Effect of white light. Plant Physiol. **44**: 1706–1711.