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REVIEW

Protein expression during *Flammulina velutipes* fruiting body formation

Yuichi Sakamoto

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Abstract Formation of the *Flammulina velutipes* fruiting body can be induced by lowering the ambient temperature (first treatment) in complete darkness. Fruiting bodies formed under these conditions elongate without pileus formation (pinhead fruiting body), suggesting that they cannot mature in complete darkness. However, after light treatment of the pinhead fruiting body (second treatment), a pileus develops immediately, and the stipe also thickens and becomes increasingly pigmented. The apical region swells as a result of cell division starting 2 days after light treatment, the pileus–stipe junction fracture and hymenium primordia form on day 4, and gills appear at day 6. Pf1 and Pf3 are specifically expressed after exposure to low temperature without light. The cell wall-associated protein [pileus-specific hydrophobin-like protein (PSH)] is specifically induced in the pileus, but not in the stipe, following the second light treatment to the pinhead fruiting body. These results suggest that Pf1 and Pf3 would be involved in fruiting body induction and that PSH would be involved in pileus formation. These phenomena will aid further histological and molecular biological investigations into the mechanisms behind fruiting body development in *F. velutipes*.

Keywords Light · Pinhead fruiting body · Pileus formation · Stipe elongation

Y. Sakamoto (✉)
Iwate Biotechnology Research Center,
22-174-4 Narita, Kitakami, Iwate 024-0003, Japan
e-mail: sakamoto@ibrc.or.jp

Introduction

Fruiting body induction of basidiomycete mushrooms is stimulated by environmental conditions such as changes in temperature or light. In many species, light is required for fruiting body induction or for other developmental stages, and complete darkness does not induce fruiting body or normal maturation. For example, light stimulates formation of the fruiting body primordium of *Favolus arcularius* (Batsch) Fr. (Kitamoto et al. 1968), *Schizophyllum commune* Fr. (Perkins and Gordon 1969), *Coprinus cinereus* (Schaeff.) Gray (Tusué 1969), and *Lentinula edodes* (Berk) Pegler (Mohamed et al. 1992a,b). It also stimulates pileus formation in *F. arcularius* (Kitamoto et al. 1974), *C. cinereus* (Kamada et al. 1978; Kamada and Tuji 1979), and *L. edodes* (Mohamed et al. 1992a,b). Furthermore, light induces other morphological changes during the life cycle of basidiomycete fungi, such as those that take place during asexual sporulation (Kües et al. 1998; Kertesz-Chaloupkova et al. 1998). In addition to fruiting body induction by light without a temperature change in *S. commune*, light also regulates several dikaryon-specific genes involved in fruiting body formation (Yli-Mattila 1987; Wessels et al. 1987; Ruiters et al. 1988). However, other environmental factors that stimulate gene expression involved in fruiting body induction of basidiomycete mushrooms have not been well described. *Flammulina velutipes* (Curtis) Singer, known as winter mushroom, is one of the most popular edible mushrooms in Japan, and it forms fruiting bodies under low temperature (Ingold 1980). Furthermore, *F. velutipes* can form fruiting bodies in complete darkness, although they elongate without pileus formation to produce a pinhead fruiting body (Plunkett 1956; Aschan-Åberg 1960; Kinugawa and Furukawa 1965; Kinugawa 1977). Therefore, the gene expression profile

involved in fruiting body induction of *F. velutipes* is considered to be different from those of mushrooms whose fruiting bodies are light induced without a temperature lowering. Although several studies describe genes that are specifically expressed in the *F. velutipes* fruiting body (Azuma et al. 1996; Kim et al. 1999; Ando et al. 2001; Yamada et al. 2005), little is known about gene expression involved in fruiting body development of *F. velutipes* at low temperature in complete darkness.

Stipe length, pileus size, and fruiting body pigmentation of *F. velutipes* are important from a commercial point of view, but the mechanisms behind stipe elongation, pileus formation, and fruiting body pigmentation are not well understood. It was shown that the diameter of the pileus increases in proportion to light intensity (up to 100 lx; Inatomi et al. 2001), and thus it is believed that *F. velutipes* pileus formation is stimulated by light. Fruiting in darkness has also been reported for several other basidiomycete mushrooms. For example, when *C. cinereus* is grown in complete darkness, it forms fruiting bodies with a long stipe and a tiny, undeveloped pileus on top that is termed a dark stipe (Tusué 1969). In addition, when light-induced fruiting body primordia of *F. arcularius* are transferred into darkness, their pilei do not develop, resulting in fruiting bodies consisting of a long stipe (Fukutomi et al. 1982; Kitamoto et al. 1999) that is termed an epilete stipe (Kitamoto et al. 1968). However, when the dark stipe of *C. cinereus* and the epilete stipe of *F. arcularius* are exposed to light, both can form a pileus at the apical region (Tusué 1969; Fukutomi et al. 1982). Recently, *F. velutipes* fruiting body induction in low temperatures and complete darkness, and fruiting body maturation after second light stimulation, have been investigated (Sakamoto et al. 2004, 2007). The expression of proteins involved in *F. velutipes* fruiting body induction and maturation was also investigated (Sakamoto et al. 2001, 2002, 2007). In this article, the relationship between protein expression and morphological changes under light and dark conditions is discussed.

Morphological changes after exposure to low temperature in complete darkness

The first changes in *F. velutipes* hyphae are observed 7 days after fruiting treatment (lowering temperature with light stimulation) by scanning electron microscopy (Azuma et al. 1996). *Flammulina velutipes* forms young fruiting bodies at 14 days and mature fruiting bodies (with gills in their pileus) at 21 days after fruiting treatment (Sakamoto et al. 2002). Young fruiting bodies develop under lowered temperature (a transfer from 23 to 16°C) in complete darkness (low-temperature treatment), and the stipe elongates without pileus formation after 21 days in these conditions to produce the pinhead fruiting body (Sakamoto et al. 2002; Fig. 1a). Kinugawa and Furukawa (1965) demonstrated that low temperature treatment of short duration (a minimal duration is 12 h at 15°C) induces fruiting body formation in *F. velutipes*, suggesting that low temperature is the critical factor for this process. Fruiting bodies are not formed 21 days after light treatment without lowering of temperature, suggesting that light cannot directly stimulate fruiting body induction in *F. velutipes* (Sakamoto et al. 2002, 2004). On the other hand, fruiting bodies formed at low temperature in complete darkness cannot form a pileus, suggesting that light may be involved in pileus formation. Pileus tissue is not formed when apical sections of 14-day pinhead fruiting bodies (Sakamoto et al. 2004, 2007) are formed. These findings support the previous accounts of fruiting of *F. velutipes* in complete darkness (Plunkett 1953, 1956; Aschan 1954; Aschan-Åberg 1958; Kinugawa 1977). It has been reported that young fruiting bodies of *F. velutipes* that are smaller than 0.2 mm and which have formed under light also have a spiky appearance, and that as the primordia increase in size the rudimentary pileus differentiates (Williams et al. 1985). These observations indicate that the pileus is not differentiated during the early stages of the primordium development of *F. velutipes*.

The *F. velutipes* pinhead fruiting body is similar in shape to that of *C. cinereus* after cultivation in complete darkness; the stipes on the *C. cinereus* fruiting body have been denoted as dark stipes (Tusué 1969). This observation suggests that in complete darkness, *F. velutipes* primordia can be induced and that stipes elongate. The fruiting bodies that form in complete darkness were found to be thinner than normal fruiting bodies that form during fruiting treatment (Sakamoto et al. 2002, 2004, 2007). Furthermore, these fruiting bodies are not pigmented, which supports the current understanding that the fruiting body color of commercially cultivated *F. velutipes* correlates with the extent of light exposure (Shiratori et al. 1982; Inatomi et al. 2001). These findings suggest that although low temperature is a critical factor for fruiting body induction of *F. velutipes*, fruiting bodies cannot mature in complete darkness.

Morphological changes after second light stimulation

Flammulina velutipes pinhead fruiting bodies that form after 21 days of low-temperature treatment in complete darkness are differentiated by light (second light treatment) to develop the pileus immediately at the apex (Sakamoto et al. 2004, 2007; Fig. 1b). This observation suggests that light can directly stimulate the formation of the *F. velutipes* pileus. After the second light treatment, the apical region

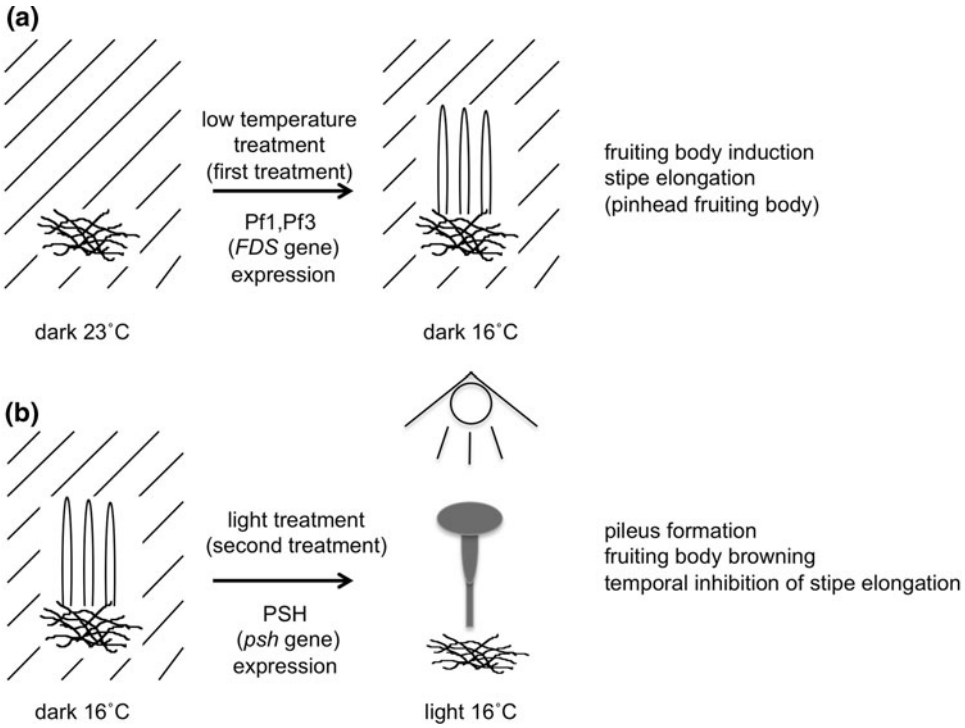

Fig. 1 Experimental scheme used to investigate the effect of temperature and light on the development of the *Flammulina velutipes* fruiting body. **a** One-month-old cultures grown in complete darkness at 23°C were then subjected to the low-temperature treatment (continuous darkness at 16°C). **b** One-month-old cultures grown in complete darkness at 23°C were then subjected to the low-temperature treatment for 21 days, at which point pinhead fruiting bodies had formed, and were then subjected to the second light treatment (continuous illumination at 16°C). *FDS Flammulina velutipes* differentiation specific gene, *PSH* pileus-specific hydrophobin

swells due to cell division to form the pileus (Sakamoto et al. 2007). In contrast, fruiting bodies that continued to be cultivated in the dark did not exhibit this development. These findings suggest that *F. velutipes* fruiting bodies that form under complete darkness have the potential to form a pileus, similar to that which has been observed for other species whose fruiting bodies can form in the dark, including *C. cinereus* (Tusué 1969) and *F. arcularius* (Fukutomi et al. 1982). Pileus tissue cannot form in the apical region of pinhead fruiting bodies that formed in the dark, and a second light treatment induced pileus tissue only at apical regions, suggesting that only these regions of pinhead fruiting bodies have the potential to develop into the pileus (Sakamoto et al. 2004, 2007). It seems that the cells that can differentiate into the pileus are established during differentiation of the fruiting body primordium. As a result, these cells should be defined as pileus primordium, which is the term used in describing pileus formation in *F. arcularius* (Fukutomi et al. 1982).

Two days after the second light treatment, neither the junction fracture between the pileus and stipe nor the hymenium has undergone clear differentiation (Sakamoto et al. 2007). The fracture and the hymenium primordium are formed at 4 days after light treatment, and it was noted that cells in the junction seemed to be attached to each other (Sakamoto et al. 2007). The fracture and the hymenium are more distinct at 6 days (Sakamoto et al. 2007). Umar and Van Griensven (1997) claimed that programmed cell death occurs during pileus formation in *Agaricus bisporus* (J.E. Lange) Imbach. In basidiomycetes, programmed cell death is observed at the meiotic stage in *C. cinereus* mutants that are defective in sporulation (Lu et al. 2003). In *F. velutipes*, dead cells (originally denoted as moribund cells) have been observed at the junction between the pileus and stipe (Williams et al. 1985). These results raise the possibility that formation of the junction fracture between the pileus and stipe is caused by programmed cell death. The junction fracture forms following the second light treatment, suggesting that light-induced programmed cell death is involved in pileus formation in *F. velutipes*. Clearly, further careful histological studies are needed to reveal the mechanism behind the junction fracture formation in *F. velutipes*.

The stipes of pinhead fruiting bodies thicken immediately after second light treatment, although thickening does not occur at the basal end of the stipe (Sakamoto et al. 2004). Sakamoto et al. (2004) showed that stipe elongation is inhibited until after 8 days of light treatment, and that stipes elongate rapidly afterward. Notably, Gruen (1969) has demonstrated that the molecule(s) that induce stipe elongation may be located in the pileus lamellae, which suggests that the pileus of *F. velutipes* may promote stipe elongation (Gruen 1976). It is considered that this rapid elongation may occur in response to the molecule(s) from the pileus. Basidiospores are not apparent 4 days after the second light treatment, but they are visible on gill surfaces at 8 days (Sakamoto et al. 2007). Kamada and Tuji (1979) have reported that fruiting body maturation, including stipe elongation, is promoted in the phase 3 pileus, in which meiosis is in progress in the basidia. Therefore, the time at

which rapid stipe elongation begins will be influenced by the stage of basidiospore development in *F. velutipes*. This stipe elongation system may ensure the effective dispersal of basidiospores. However, the stipe rapidly elongates at early stages of development in the dark, after which the elongation rate decreases (Sakamoto et al. 2004). This observation suggests that there may be another stipe elongation system that operates in the dark which does not involve the pileus-derived stipe elongation molecule(s).

Protein expression after low-temperature treatment

To investigate the influence of lowering temperature on fruiting body induction of *F. velutipes* at the molecular level, mycelial proteins expressed after low temperature were investigated by two-dimensional electrophoresis (Sakamoto et al. 2002). In all, 22 proteins are newly expressed after temperature lowering under continuous darkness (Sakamoto et al. 2002). These proteins are not induced by light, and the collective results indicate that temperature is the critical factor for fruiting body induction in *F. velutipes* (Sakamoto et al. 2002), and that many of the proteins involved in fruiting body induction in *F. velutipes* are regulated by low temperature.

Two protein spots, Pf1 and Pf3, are both expressed in mycelia after low-temperature treatment and in the fruiting body (Sakamoto et al. 2002). N-terminal amino acid sequences of Pf1 and Pf3 are highly similar to the deduced amino acid sequence of the *F. velutipes FDS* (*Flammulina velutipes* differentiation specific) gene (Azuma et al. 1996; Sakamoto et al. 2002). There is no gene similar to *FDS* except for those encoding hypothetical proteins in the *Laccaria bicolor* (Maire) P. D. Orton genome (Martin et al. 2008), and no obvious motif is found in the putative amino acid sequence encoded by *FDS*. Therefore, the molecular function of *FDS* cannot be inferred from sequence information. However, Pf1 and Pf3 are induced by temperature lowering without light stimulation, and *FDS* is transcribed abundantly in the fruiting body; therefore, it likely has an important role in fruiting body formation.

Protein expression after second light treatment

Previous studies of other basidiomycetes have investigated genes involved in pileus formation (Muraguchi and Kamada 1998) or the role of light in gene expression involved in fruiting body induction (Ruiters et al. 1988). However, the relationship between protein expression and pileus differentiation after light exposure is not yet understood. Protein expression in the pinhead fruiting body formed under complete darkness has been compared with that of the pileus and stipe of the fruiting body after the second light treatment (Sakamoto et al. 2007). One protein [pileus-specific hydrophobin-like protein (PSH)] is expressed specifically and abundantly in the pileus (especially in the gill) but not in pinhead fruiting bodies or stipes following a second light treatment (Sakamoto et al. 2007). This finding suggests that PSH expression is induced after light treatment and that it may be involved in pileus formation in *F. velutipes*. A BLAST search revealed that there are no genes similar to the PSH encoding gene (*psh*) in any database, including basidiomycete genome databases, suggesting that PSH is a novel protein. The deduced amino acid sequence of *psh* has a serine-rich motif in its central region and a hydrophobin-like motif in its C-terminal region (Sakamoto et al. 2007). Conserved cysteines of hydrophobin proteins are also present in the hydrophobin-like region of PSH, and a hydropathy plot of this region is similar to that of the corresponding region of the *F. velutipes* hydrophobin FVH1 (Sakamoto et al. 2007). There are many hydrophobin genes in fungi, and two hydrophobin genes in *F. velutipes* have been reported (Ando et al. 2001; Yamada et al. 2005). It was reported that hydrophobins SC3 and SC15 in *S. commune* interact with each other (Lugones et al. 2004). By analogy, PSH may interact with other *F. velutipes* hydrophobins to regulate pileus formation.

The expression level of *psh* in the pinhead fruiting body is low (day 0) and similar to that in the stipe of the mature fruiting body: at 8 days after second light treatment, the expression of *psh* was significantly increased compared to day 0, and the expression level is similar to that in the pileus of the mature fruiting body (Sakamoto et al. 2007). Following light treatment, the expression of *psh* increases, suggesting that it is induced by light exposure and is specifically transcribed in the pileus. The *psh* is not expressed in vegetative mycelium exposed to light, and its expression in the pileus increases only several days after light treatment (Sakamoto et al. 2007). These results suggest that *psh* is not directly regulated by light but that it may be expressed as a secondary response following light treatment.

Future photomorphological and skotomorphological studies in *F. velutipes*

This report has showed that the *F. velutipes* fruiting body can be induced in complete darkness under low temperature (skotomorphogenesis; see Fig. 1), that the fruiting body formed under this condition has an undeveloped pileus at its apex, and that the pileus forms immediately after a second light treatment (photomorphogenesis; Fig. 1). Because *F. velutipes* fruiting body formation can be induced by lowering temperature without any other

environmental factors, it is a good system for investigating low-temperature sensing for fruiting body formation. For example, a low-temperature sensor, Mga2p, was found in *Saccharomyces cerevisiae* (Nakagawa et al. 2002). Mga2p is produced as a dormant precursor that is firmly anchored to the endoplasmic reticulum or nuclear envelope membrane by its C-terminal region. In response to a low-temperature signal, its N-terminal transcription factor domain is released into the cytosol by ubiquitin/proteasome-dependent processing and activates cold tolerance proteins such as *OLE1* (Nakagawa et al. 2002). Mga2 homologues are found in basidiomycete genomes and are likely to have similar functions in these fungi. For example, the *Cryptococcus neoformans* Mga2 homologue is involved in high-temperature growth (Kraus et al. 2004). *Flammulina velutipes* is a suitable basidiomycete for investigating cold sensors such as Mga2 for fruiting body induction.

Fruiting bodies of basidiomycetes also respond to light in an effective strategy for spore diffusion. In this report, it is shown that pileus formation in *F. velutipes* can be induced by second light treatment; therefore, *F. velutipes* is suitable for investigating pileus formation and stipe elongation. Separation between the pileus and stipe may be caused by programmed cell death (Williams et al. 1985; Sakamoto et al. 2007). Therefore, further studies should reveal the potential relationships between light sensing and pileus formation caused by programmed cell death. It has also been shown that a second light treatment induces fruiting body browning, stipe thickening, and temporal inhibition of stipe elongation (Sakamoto et al. 2004). Several molecules involved in stipe elongation have been reported (Gruen 1969; Hirai et al. 1998), and these are relevant for investigating fruiting body development in *F. velutipes* under light and dark conditions.

Photomorphogenesis in basidiomycetes was discussed by Eger-Hummel (1980), and it was shown that fruiting body differentiation in these fungi is regulated by blue light. Putative blue light receptor genes that are homologous to *Neurospora crassa wc-1* and *wc-2* (Ballario et al. 1996) were cloned from *C. cinereus* (Terashima et al. 2005) and *L. edodes* (Sano et al. 2008, 2009). In addition, it was shown that the mutation in the light response-defective mutant of *C. cinereus*, dst1, maps to a WC1 homologue (Terashima et al. 2005). The *L. edodes* WC1/WC2 homologues PHRA and PHRB interact with each other and have a DNA-binding ability similar to that of the *N. crassa* WC1/WC2 complex (Sano et al. 2009). These results indicate that blue light receptor(s) regulate fruiting body differentiation in mushrooms. It has been found that *F. velutipes* pileus formation is induced by blue light wavelengths (Aschan-Åberg 1960). Also, we observed that *F. velutipes* pileus formation is induced by blue light but not by red or yellow light (Sakamoto et al. 2007). This finding suggests that *F. velutipes* pileus formation is also regulated by blue light, and further study of blue light sensing in *F. velutipes* will be helpful for understanding fruiting body maturation.

Several basidiomycete genomes have been sequenced (Martin et al. 2008), including those of *L. bicolor*, *C. cinereus*, and *S. commune*. Reverse genetic analysis techniques, including gene disruption and RNA interference, have been developed for several basidiomycetes, such as *C. cinereus* (Namekawa et al. 2005) and *A. bisporus* (Eastwood et al. 2008). Genome sequence data and reverse genetic methods will be available for *F. velutipes* in the near future, thereby facilitating functional analysis. The findings described in this report will provide a basis for further molecular and genetic analyses of skoto- and photomorphological phenomena in *F. velutipes*.

As discussed here, *F. velutipes* fruiting bodies develop in complete darkness, and these fruiting bodies have a long stipe and an undeveloped pileus at the apex. Furthermore, fruiting body maturation, including pileus formation and stipe thickening, elongation, and pigmentation, can be induced by a second light treatment. These phenomena will aid further histological and molecular biological investigations into the mechanisms behind fruiting body development in *F. velutipes*.

References

Ando A, Harada A, Miura K, Tamai Y (2001) A gene encoding a hydrophobin, *fvh1*, is specifically expressed after the induction of fruiting in the edible mushroom *Flammulina velutipes*. Curr Genet 39:190–197

Aschan K (1954) The production of fruiting bodies in *Collibia velutipes* I. Influence of different culture conditions. Physiol Plant 7:571–591

Aschan-Åberg K (1958) The production of fruiting bodies in *Collybia velutipes* II. Further studies on the influence of different culture conditions. Physiol Plant 11:312–328

Aschan-Åberg K (1960) The production of fruit bodies in *Collybia velutipes* III. Influence of the quality of light. Physiol Plant 13:276–279

Azuma T, Harada A, Kim D, Sakuma Y, Kojima Y, Miura K (1996) Isolation of a gene specifically expressed during fruiting body differentiation in *Flammulina velutipes*. Mokuzai Gakkaishi 42:688–692

Ballario P, Vittorioso P, Magrelli A, Talora C, Cabibbo A, Macino G (1996) White collar 1, a central regulator of the blue light responses in *Neurospora*, is a zinc-finger protein. EMBO J 15:1650–1657

Eastwood DC, Challen MP, Zhang C, Jenkins H, Henderson J, Burton KS (2008) Hairpin-mediated down-regulation of the urea cycle enzyme argininosuccinate lyase in *Agaricus bisporus*. Mycol Res 112:708–716

Eger-Hummel G (1980) Blue light photomorphogenesis in mushroom (*basidiomycetes*). In: Senger H (ed) Blue-light syndrome. Springer, New York, pp 555–562

Fukutomi M, Horikoshi T, Akai S, Kitamoto Y (1982) Ultrastructural aspects of photoinduced pileus formation in *Favolus arcularius* I. Pileusu-primodium formation. Trans Mycol Soc Jpn 23:1–12

Gruen HE (1969) Growth, rotation of *Flammulina velutipes* fruiting bodies and the dependence of stipe elongation on the cap. Mycologia 61:149–166

Gruen HE (1976) Promotion of stipe elongation in *Flammulina velutipes* by a diffusate from excised lamellae supplied with nutrients. Can J Bot 54:1306–1315

Hirai Y, Ikeda M, Murayama T, Ohata T (1998) New monoterpentriols from the fruiting body of *Flammulina velutipes*. Biosci Biotechnol Biochem 62:1364–1368

Inatomi S, Namba K, Kodaira R, Okazaki M (2001) Effects of light exposure at different cultivation process for the production of fruiting bodies in a colored strain "NAKANO" of *Flammulina velutieps*. Mushroom Sci Biotechnol 9:21–26

Ingold CT (1980) *Flammunina velutipes*. Trans Br Mycol Soc 14:112–118

Kamada T, Tuji M (1979) Darkness-induced factor affecting basidiocarp maturation in *Coprinus macrorhizus*. Plant Cell Physiol 20:1445–1448

Kamada T, Kurita R, Takemaru T (1978) Effects of light on basidiocarp maturation in *Coprinus macrorhizus*. Plant Cell Physiol 19:263–275

Kertesz-Chaloupkova K, Walser PJ, Granado JD, Aebi M, Kües U (1998) Blue light overrides repression of asexual sporulation by mating type genes in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 23:95–109

Kim D, Azuma T, Harada A, Sakuma Y, Ando A, Tamai Y, Miura K (1999) Cloning and sequence analysis of a cDNA for the gene *FVFD30*, specifically expressed during fruiting body development in *Flammulina velutipes*. Mushroom Sci Biotechnol 7:95–99

Kinugawa K (1977) *Collybia velutipes* can fruiting under total darkness. Trans Mycol Soc Jpn 18:353–356

Kinugawa K, Furukawa H (1965) The fruiting body formation in *Collybia velutipes* induced by the lower temperature treatment of one short duration. Bot Mag Tokyo 78:240–244

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

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

Kitamoto Y, Akita K, Horikoshi T (1999) 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*. Mycoscience 40:103–108

Kraus PR, Boily MJ, Giles SS, Stajich JE, Allen A, Cox GM, Dietrich FS, Perfect JR, Heitman J (2004) Identification of *Cryptococcus neoformans* temperature-regulated genes with a genomic-DNA microarray. Eukaryot Cell 3:1249–1260

Kües U, Granado JD, Hermann R, Boulianne RP, Kertesz-Chaloupkova K, Aebi M (1998) The A mating type and blue light regulate all known differentiation processes in the basidiomycete *Coprinus cinereus*. Mol Gen Genet 260:81–91

Lu BC, Gallo N, Kües U (2003) White-cap mutants and meiotic apoptosis in the basidiomycete *Coprinus cinereus*. Fungal Genet Biol 39:82–93

Lugones LG, de Jong JF, de Vries OMH, Jalving R, Dijksterhuis J, Wosten HAB (2004) The SC15 protein of *Schizophyllum commune* mediates formation of aerial hyphae and attachment in the absence of the SC3 hydrophobin. Mol Microbiol 53:707–716

Martin F, Aerts A, Ahrén D, Brun A, Danchin EG, Duchaussoy F, Gibon J, Kohler A, Lindquist E, Pereda V, Salamov A, Shapiro HJ, Wuyts J, Blaudez D, Buée M, Brokstein P, Canbäck B, Cohen D, Courty PE, Coutinho PM, Delaruelle C, Detter JC, Deveau A, DiFazio S, Duplessis S, Fraissinet-Tachet L, Lucic E, Frey-Klett P, Fourrey C, Feussner I, Gay G, Grimwood J, Hoegger PJ, Jain P, Kilaru S, Labbé J, Lin YC, Legué V, Le Tacon F, Marmeisse R, Melayah D, Montanini B, Muratet M, Nehls U, Niculita-Hirzel H, Oudot-Le Secq MP, Peter M, Quesneville H, Rajashekar B, Reich M, Rouhier N, Schmutz J, Yin T, Chalot M, Henrissat B, Kües U, Lucas S, Van de Peer Y, Podila GK, Polle A, Pukkila PJ, Richardson PM, Rouzé P, Sanders IR, Stajich JE, Tunlid A, Tuskan G, Grigoriev IV (2008) The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature (Lond) 452:88–92

Mohamed AB, Meguro S, Kawachi S (1992a) The effects of light on primordia and fruiting body formation of *Lentinus edodes* in a liquid medium. Mokuzai Gakkaishi 38:600–604

Mohamed AB, Meguro S, Kawachi S (1992b) The effects of light on primordia and fruiting body formation of *Lentinus edodes* in a liquid medium II. The effects of the duration of illumination on primordium formation. Mokuzai Gakkaishi 38:876–879

Muraguchi H, Kamada T (1998) The *ich1* gene of the mushroom *Coprinus cinereus* is essential for pileus formation in fruiting. Development (Camb) 125:3133–3141

Nakagawa Y, Sakumoto N, Kaneko Y, Harashima S (2002) Mga2p is a putative sensor for low temperature and oxygen to induce OLE1 transcription in *Saccharomyces cerevisiae*. Biochem Biophys Res Commun 291:707–713

Namekawa SH, Iwabata K, Sugawara H, Hamada FN, Koshiyama A, Chiku H, Kamada T, Sakaguchi K (2005) Knockdown of LIM15/DMC1 in the mushroom *Coprinus cinereus* by double-stranded RNA-mediated gene silencing. Microbiology 51:3669–3678

Perkins JH, Gordon SA (1969) Morphogenesis in *Schizophyllum commune* II. Effects of monochromatic light. Plant Physiol 44:1712–1716

Plunkett BE (1953) Nutritional and other aspects of fruiting body production in pure cultures of *Collybia velutipes* (Cur.) Fr. Ann Bot (NS) 17:193–217

Plunkett BE (1956) The influences of factors of the aeration complex and light upon fruiting body form in pure cultures of an agaric and a polypore. Ann Bot (NS) 20:563–585

Ruiters MHJ, Sietsma JH, Wessels JGH (1988) Expression of dikaryon-specific mRNAs of *Schizophyllum commune* in relation to incompatibility genes, light, and fruiting. Exp Mycol 12:60–69

Sakamoto Y, Ando A, Tamai Y, Miura K, Yajima T (2001) Differential protein expression in the fruiting dikaryon and the non-fruiting monokaryon of *Flammulina velutipes*. Mycol Res 105:177–182

Sakamoto Y, Ando A, Tamai Y, Miura K, Yajima T (2002) Protein expressions during fruiting body induction of *Flammulina velutipes* under reduced temperature. Mycol Res 106:222–227

Sakamoto Y, Ando A, Tamai Y, Yajima T (2004) Influences of temperature and light on morphological changes during fruit body formation in *Flammulina velutipes*. Mycoscience 45:333–339

Sakamoto Y, Ando A, Tamai Y, Yajima T (2007) Pileus differentiation and pileus-specific protein expression in *Flammulina velutipes*. Fungal Genet Biol 44:14–24

Sano H, Narikiyo T, Kaneko S, Yamazaki T, Shishido K (2008) Sequence analysis and expression of a blue-light photoreceptor gene, Le.phrA, from the basidiomycetous mushroom *Lentinula edodes*. Biosci Biotechnol Biochem 71:2206–2213

Sano H, Kaneko S, Sakamoto Y, Sato T, Shishido K (2009) The basidiomycetous mushroom *Lentinula edodes* white collar-2 homolog PHRB, a partner of putative blue-light photoreceptor

PHRA, binds to a specific site in the promoter region of the *L. edodes* tyrosinase gene. Fungal Genet Biol 46:333–341

Shiratori T, Kakimoto Y, Nakamura K (1982) The coloring of bottle cultured *enokitake* (in Japanese). Bull Nagano Veg Ornam Crops Exp Jpn 2:89–98

Terashima K, Yuki K, Muraguchi H, Akiyama M, Kamada T (2005) The *dst1* gene involved in mushroom photomorphogenesis of *Coprinus cinereus* encodes a putative photoreceptor for blue light. Genetics 171:101–108

Tusué YM (1969) Experimental control of fruiting body formation in *Coprinus macrorhizus*. Dev Growth Differ 11:164–178

Umar MH, Van Griensven LJ (1997) Morphogenetic cell death in developing primordia of *Agaricus bisporus*. Mycologia 89:274–277

Wessels JGH, Mulder GH, Springer J (1987) Expression of dikaryon-specific and non-specific mRNAs of *Schizophyllum commune* in relation to environmental conditions and fruiting. J Gen Microbiol 133:2557–2561

Williams MA, Bekkett A, Read ND (1985) Ultrastructural aspects of fruiting body differentiation in *Flammulina velutipes*. In: Moore D, Casselton LA, Wood DA, Frankland JC (eds) Developmental biology in higher fungi. The British Mycological Society. Cambridge University Press, Cambridge, pp 429–450

Yamada M, Sakuraba S, Shibata K, Inatomi S, Okazaki M, Shimosaka M (2005) Cloning and characterization of a gene coding for a hydrophobin, Fv-hyd1, specifically expressed during fruiting body development in the basidiomycete *Flammulina velutipes*. Appl Microbiol Biotechnol 67:240–246

Yli-Mattila T (1987) The effect of UV-A light on cAMP level in the basidiomycete *Schizophyllum commune*. Physiol Plant 69:451–455