Mycoscience (2009) 50:331–342
DOI 10.1007/s10267-009-0489-x

© The Mycological Society of Japan and Springer 2009

FULL PAPER

Takehito Nakazawa · Shinya Kaneko · Hitoshi Murata
Takashi Kamada · Kazuo Shishido

The homologue of *Lentinula edodes ctg1*, a target for CDC5 and its interacting partner CIPB, from *Coprinopsis cinerea* is involved in fruiting-body morphogenesis of *C. cinerea*

Received: January 8, 2009 / Accepted: January 30, 2009

Abstract We have previously isolated a target gene for the *Lentinula edodes* CDC5 (Le.CDC5) containing a c-Myb-type DNA-binding domain. The gene, termed *ctg1*, contains a 7-bp Le.CDC5-binding sequence (5′-GCAATCT3′) in its transcribed region downstream of the start codon and 6-bp CIPB-binding sequences in the 5′-upstream and transcribed regions. The expression of *ctg1* appeared to be cooperatively regulated by Le.CDC5 and CIPB. Here, we found that the gene expression regulation system of *ctg1*–CDC5–CIPB is conserved in the basidiomycetous mushroom *Coprinopsis cinerea*. Similar to *L. edodes*, the Le.CDC5 homologue (named Cc.Cdc5) bound to a 7-bp sequence, 5′-GCAAGCT-3′, in the transcribed region of the *ctg1* homologue (*Cc.ctg1*) and the CIPB homologue (Cc.CipB) to 6-bp sequences in its 5′-upstream and transcribed regions. To study the biological function of the *Cc.ctg1* gene (and also the *ctg1* gene) in *C. cinerea*, we bred the *C. cinerea* homokaryotic fruiting strains, consistently producing a large amount of the *Cc.ctg1* (or *ctg1*) transcript. Analysis of the phenotype of these strains suggested that the *Cc.ctg1* gene (or the *ctg1* gene) is mainly involved in the regulation of stipe elongation.

Key words *Coprinopsis cinerea* · Fruiting · *Lentinula edodes* · Transcriptional regulation

T. Nakazawa (✉) · S. Kaneko · K. Shishido
Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Yokohama, Kanagawa 226-8501, Japan
Tel. +81-45-924-5716; Fax +81-45-924-5773
e-mail: tnakazaw@bio.titech.ac.jp

H. Murata
Department of Applied Microbiology and Mushroom Science, Forestry and Forest Product Research Institute, Ibaraki, Japan

T. Kamada
Department of Biology, Faculty of Science, Okayama University, Okayama, Japan

Introduction

Basidiomycetous mushrooms exhibit a dramatic morphological differentiation from vegetative mycelia to huge fruiting bodies in which a large number of basidiospores are produced. To investigate the molecular mechanism underlying the fruiting development of the basidiomycetous mushroom *Lentinula edodes* (Berk.) Pegler, commonly called "shiitake," a variety of genes (or cDNAs) specific for fruiting development have been isolated from *L. edodes* (Leung et al. 2000; Miyazaki et al. 2005; Chum et al. 2008), and their properties and functions have been analyzed (Lee et al. 2007; Nakazawa et al. 2008a). One of these, the *Le.cdc5* gene (Miyazaki et al. 2004), is a homologue of the *Schizosaccharomyces pombe cdc5*$^+$ gene (Ohi et al. 1994). The deduced *Le.cdc5* product (Le.CDC5) consists of 842 amino acid (hereafter designated as aa) residues. In order, starting from the N-terminus, it possesses a c-Myb-type DNA-binding domain, several putative nuclear localization signals, putative transactivation domains such as proline-rich and acidic aa-rich regions, two phosphorylation sites for A kinase, and a leucine zipper $LX_6LX_6LX_6L$ between the A-kinase-phosphorylation sites (Miyazaki et al. 2004). The N-terminal 185-aa peptide of Le.CDC5 [named Le.CDC5 (1–185) peptide for short], which is homologous to the c-Myb-type DNA-binding domain, binds to a 7-bp sequence with the consensus sequence 5′-GCAATGT-3′. So far, *S. pombe cdc5*$^+$ gene product (Sp.cdc5p)-related proteins (SPCDC5RPs) such as *Arabidopsis thaliana* CDC5 and human Cdc5 proteins have been shown to bind to a 7-bp sequence with the consensus sequence 5′-CTCAGCG-3′ (complementary, 5′-CGCTGAG-3′) (Hirayama and Shimosaki 1996) and to a 12-bp sequence with the consensus sequence 5′-GATTTAACATAA-3′ (complementary, 5′-TTATGTTAAATC-3′) (Lei et al. 2000), respectively, and to activate transcription (Bernstein and Coughlin 1998; Lin et al. 2007). These findings suggest that they are transcription regulators. However, no target gene was identified. Sp.cdc5p, *Saccharomyces cerevisiae* Cef1 (Sp.cdc5p homologue), and human Cdc5L were later found to be essential

for pre-mRNA splicing and to represent a component of large splicing complexes (Burns et al. 1999; McDonald et al. 1999; Tsai et al. 1999; Ajuh et al. 2000). To demonstrate that the Le.CDC5 protein functions as a transcription factor, we previously attempted to isolate its target gene(s). A genomic binding site (GBS) cloning experiment (Inoue et al. 1991) with the Le.CDC5 (1–185) peptide and an inverse polymerase chain reaction (PCR) resulted in the isolation of a target gene designated *ctg1* (CDC5 target gene) (Nakazawa et al. 2008a). It contains a 7-bp consensus-like sequence (5′-GCAATCT-3′) for Le.CDC5 binding in its transcribed region downstream of the start codon. Le.CDC5 was found to interact with a novel protein, CIPB (CDC5 interacting partner B; 127 aa residues), which binds to a 6-bp sequence with the consensus sequence 5′-CAACAC/T/G-3′. The *ctg1* gene contains nine 6-bp consensus (or consensus-like) sequences; six are in the 5′-upstream region and three in the transcribed region downstream of the start codon. At least two each of the upstream and downstream sequences appear to bind CIPB in vitro. These findings suggest that Le.CDC5 and CIPB can cooperatively regulate the expression of *ctg1* (Nakazawa et al. 2008a). The *ctg1* gene product (CTG1; 159 aa residues) contains a short leucine zipper-like motif, $LALX_5LX_6L$, at the N-terminus and a short basic amino acid sequence, KKK, near the C-terminus. Northern blot analysis suggested that the *ctg1* gene is transcribed most actively in the upper halves of the stipes of fruiting bodies, implying its involvement in regulation of stipe elongation (Nakazawa et al. 2008a).

In this article, our computer analysis showed that the homologues of *Le.cdc5*, *ctg1*, and *cipB* are present in *Coprinopsis cinerea* (Schaeff.) Redhead. As in *L. edodes*, the expression of *C. cinerea ctg1* (*Cc.ctgt1*) seemed likely to be cooperatively regulated by *C. cinerea cdc5* product (Cc. Cdc5) and *C. cinerea cipB* (Cc.CipB) product in *C. cinerea*. *Cc.ctg1* was suggested to be mainly involved in the regulation of stipe elongation of *C. cinerea*.

Materials and methods

Coprinopsis cinerea strains and culture conditions

Coprinopsis cinerea strain #326 (*AmutBmut pab1-1* / homokaryotic fruiting; = JCM 15979, Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Center, Saitama, Japan) (Pukkila and Casselton 1991) and its *dst1* mutant Bm1 (*AmutBmut dst1–4* / a REMI mutant from #326; = JCM 15980) obtained by restriction enzyme-mediated integration (REMI) using the plasmid pPHT1 and the restriction enzyme *Bam*HI as described in Cummings et al. (1999) and Makino and Kamada (2004) were used in this study. The *dst1* gene (dark stipe 1) encodes a putative photoreceptor for blue light and is essential for photomorphogenesis of *C. cinerea* (Terashima et al. 2005). They were cultured on MYG solid medium [1% (w/v) malt extract, 0.4 % (w/v) glucose, 0.4 % (w/v) yeast extract] containing 1.5% agar at 25ºC under a 12-h white light/12-h dark regime to form fruiting bodies.

GST-pulldown analysis

The cDNA fragment containing *Cc.cdc5* at nucleotides 1701–2469 (hereafter abbreviated as *Cc.cdc5*, cDNA fragment nt 1701–2469) (DDBJ/EMBL/GenBank accession no. AB465734), encoding the Cc.Cdc5 peptide at aa residues 568–822 [hereafter abbreviated as the Cc.Cdc5 (aa 568–822) peptide] containing a leucine zipper, and the *Cc.cipB* cDNA fragment (nt 1–378) (accession no. AB465736), encoding the Cc.CipB (aa 1–126) peptide (prepared by PCR using *Cc.cdc5* forward primer (hereafter abbreviated as "f") 1 and *Cc.cdc5* reverse primer (hereafter abbreviated as "r") 1 and *Cc.cipB* f1 and *Cc.cipB* r1 (Table 1) were inserted into the expression vector pGEX-2TK (GE Healthcare, Buckinghamshire, UK). The resulting plasmids were introduced into *Escherichia coli* BL21 (GE Healthcare), producing the glutathione *S*-transferase (GST)-fused proteins GST-Cc. Cdc5 (aa 568–822) and GST-Cc.CipB (aa 1–126) (in this article, the foregoing abbreviations were used for all the fragments of cDNA and protein). To produce the hemagglutinin (HA)-tagged Cc.Cdc5 peptide as prey, we used the expression vector pET-HA constructed as follows: the 48-bp fragment encoding an HA epitope tag was isolated by digesting pGADT7 (Clontech, Mountain View, CA, USA) with *Nco*I and then inserted into pET22b (Novagen, Darmstadt, Germany). The *Cc.cdc5* cDNA fragment (nt 1701–2469) was inserted into pET-HA and the resulting plasmid was introduced into *E. coli* BL21 DE3 (plysS) (Novagen), producing HA-Cc.Cdc5 (aa 568–822) as a prey protein. The GST-pulldown assay was performed according to the procedure reported previously (Nakazawa et al. 2008a). The pulled-down HA-Cc.Cdc5 (568–822) was detected by immunoblotting using anti-HA-antibody F-7 (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Electrophoretic mobility shift analysis (EMSA)

Two DNA fragments [160-bp *Cc.ctg1* fragment (nt (-)162–(-)3), probe 1, and 169-bp *Cc.ctg1* fragment (nt 493–661), probe 2] were prepared by *Cc.ctg1* f3 and *Cc.ctg1* r3, *Cc.ctg1* f4, and *Cc.ctg1* r4, respectively (see Fig. 4A, later in this article; also see Table 1). The sequence of the *Cc.ctg1* gene can be found in the *C. cinerea* genome database (contig 242: 120820–122052). The two fragments were digested with *Hin*dIII (for probe 1) or *Xho*I (for probe 2), respectively, and the digested fragments were end labeled with [α^{32}P]dCTP and Klenow fragment as described by Miyazaki et al. (2004). GST-Cc.Cdc5 (aa 1–200) was prepared as follows: the *Cc.cdc5* cDNA sequence (nt 1–600) encoding the Cc.Cdc5 peptide (aa 1–200) with the c-Myb-type DNA-binding domain (prepared by PCR using *Cc.cdc5* f2 and *Cc.cdc5* r2; see Table 1) was inserted into pGEX-2TK and the resulting recombinant plasmid was introduced into *E. coli* BL21, producing the GST-fused Cc.Cdc5 (aa 1–200). EMSA was performed according to Miyazaki et al. (2004). Where necessary, nonlabeled DNA fragments were also incubated as a competitor in the amounts indicated in the legend to Fig. 4 (see later in article).

Table 1. Primers used in this study

Primer	Sequence (5′–3′)
β*1-tub* promoter forward	TGGTTTAAGCTT(*Hin*dIII)CATTTTGGTGGCCC
β*1-tub* promoter reverse	TTTCAGATATC(*Eco*RV)CTGGGAACGCGAGGTCAGC
β*1-tub* terminator forward	AACTGATGATTCGCGCGATTC
β*1-tub* terminator reverse	TGATATCGGTCCACAATATTCATCTCTCCATCG
β*1-tub* intron forward	ACCTCGATATC(*Eco*RV)GGCCAGGTCTGTCC
β*1-tub* intron reverse	AACCTCCCAGAATTC(*Eco*RI)GGCACCTATAAG
β*1-tub* qRT-PCR forward	AACAACTGGGCCAAAGGACATACAC
β*1-tub* qRT-PCR reverse	TGTAAGGCTCAACGACGGTATGG
pP[x]EGFP forward	TGAGCCTGAATTC(*Eco*RI)CCTCCATCGCACCCCGAC
pP[x]EGFP reverse	TTAGTTTGCCCAGCATTGATTGGTC
pCOE2 qRT-PCR forward	TGCGGTAACCAAATCGGTGCC
Cc.cdc5 forward 1	TGCTCAATTGATTTATGCGGAGATGG
Cc.cdc5 forward 2	TCTCTCACCTGAACGGGATCC(*Bam*HI)ATGGTCAATAATC
Cc.cdc5 forward 3	TCTCTCACCTGAACGAGCATCATGG
Cc.cdc5 reverse 1	CTACGAGTCCTCCTCCATGGCAGC
Cc.cdc5 reverse 2	ACCAGCAGCCTTGAATTC(*Eco*RI)ACGCTTC
Cc.cdc5 reverse 3	AGGAGTTTCTCATCTTCTGTTTTGGAC
Cc.cipB forward 1	TGCACGGATCC(*Bam*HI)ATGTCCTCCACCCTCCTCC
Cc.cipB forward 2	ATGTCCTCCACCCTCCTCCG
Cc.cipB reverse 1	AATCTAGAATTC(*Eco*RI)AGTGGTGTGCGGGCTTGGGGAC
Cc.cipB reverse 2	TACGTGAGCATCCTTGGCGAC
Cc.cipB reverse 3	CTAGTGGTGTGCGGGCTTGG
Cc.ctg1 forward 1	AGGGCGACTAGTACGAGCAGGG
Cc.ctg1 forward 2	GATGACCCCTATCAGTGCCTTCTC
Cc.ctg1 forward 3	TCGTCTCACACCTCGAGATCTTTTCATCTTCC
Cc.ctg1 forward 4	TGCGTTCTCGAG(*Xho*I)CTCCCACTCCCCACG
Cc.ctg1 forward 5	ACGCCGTGTCCCCGATTACTTCC
Cc.ctg1 reverse 1	TCATTCTGGTTAGAATTTAAACCGTCGTCG
Cc.ctg1 reverse 2	TATCCATCCTCATCTCCCAACCGAAC
Cc.ctg1 reverse 3	AGGGGTAAGCTT(*Hin*dIII)CGTCGTAGTACCGG
Cc.ctg1 reverse 4	TGTGTGCTCTCGAG(*Xho*I)GCGCTGGCGAGCGTG
Cc.ctg1 reverse 5	AACACGATATC(*Eco*RV)TTGAATTCTCCTCGATCAGC
ctg1 reverse	TGGTCATGAGGAGTGGAAGTGC
M13 reverse	ACAATTTCACAGGAAACAGCTATGACC

Construction of plasmids used for the transformation of *C. cinerea*

Vectors pCOE1 and pCOE2 (Fig. 1) were constructed basically according to Cummings et al. (1999) and Namekawa et al. (2005). Three fragments containing *C. cinerea* β*1-tubulin* gene (*tub1*) promoter [nt (-)433–(-)1], terminator (nt 1959–2371), and 5′-portion (nt 27–193) (27th bases of 1st exon–5th bases of 3rd exon) were obtained by PCR using β*1-tub* promoter f and β*1-tub* promoter r, β*1-tub* terminator f and β*1-tub* terminator r, and β*1-tub* intron f and β*1-tub* intron r, respectively (see Table 1). The sequence of the β*1-tub* gene can be found in contig 224:125 488–127 424 in the *C. cinerea* genome database (http://www.broad.mit.edu/annotation/genome/coprinus_cinereus/Home.html).

For construction of pCOE1, the β*1-tub* promoter-containing fragment was digested with *Hin*dIII and *Eco*RV and inserted into pBluescript-SK+ (Toyobo, Osaka, Japan). The resulting plasmid was digested with *Sac*I, and the generated ends were blunted with T4 DNA polymerase (Toyobo); the β*1-tub* terminator-containing fragment was inserted into it at the blunt ends. For pCOE2, the β*1-tub* promoter-containing and 5′-portion-containing fragments were digested with *Hin*dIII and *Eco*RV and with *Eco*RI and *Eco*RV, respectively. These fragments were inserted into pBluescript-SK+. The resulting plasmid was digested with *Spe*I and the generated ends were blunted; the β*1-tub* terminator-containing fragment was fused to the blunt ends. The 5′-portion of β*1-tub* contains two introns, which are required for efficient production of the expressed products of intronless genes in basidiomycetous mushrooms (Lugones et al. 1999; Burns et al. 2005; Yamazaki et al. 2006). The plasmids pCOE1-Cc.ctg1 and pCOE2-ctg1 used for overexpression of *Cc.ctg1* and *ctg1* in *C. cinerea* were constructed as follows. pCOE1-Cc.ctg1: the *Cc.ctg1* fragment [nt (-)1–1232], which corresponds to Contig 242: 120 820–122 052 in the *C. cinerea* genome database) was amplified by PCR using *Cc.ctg1* f2 and *Cc.ctg1* r2, and was inserted into the *Eco*RV site of pCOE1. pCOE2-ctg1: pCOE2 was digested with *Eco*RI and the generated ends were blunted. To the blunt ends the *ctg1* fragment (nt 1–477) (accession no. BAF76763) was fused (see Fig. 7A later in this article).

Plasmids pP[x]EGFP and pLTR-hph (Fig. 1) were constructed as follows. pP[x]EGFP: the fragment containing the EGFP (efficient green fluorescent protein) gene (*egfp*) (nt 1–717) (accession no. AAB08058) and the *Nos* terminator was obtained from the vector pHHM191 by PCR using pP[x]EGFP f and pP[x]EGFP r (see Table 1). This fragment was digested with *Eco*RI and inserted into pBluescript-SK+. pHHM191 was constructed from pHHM192 (Murata et al. 2006); the *DsRed2* sequence of pHHM192 was replaced with the *egfp* sequence. pLTR-hph (hygromycin B resis-

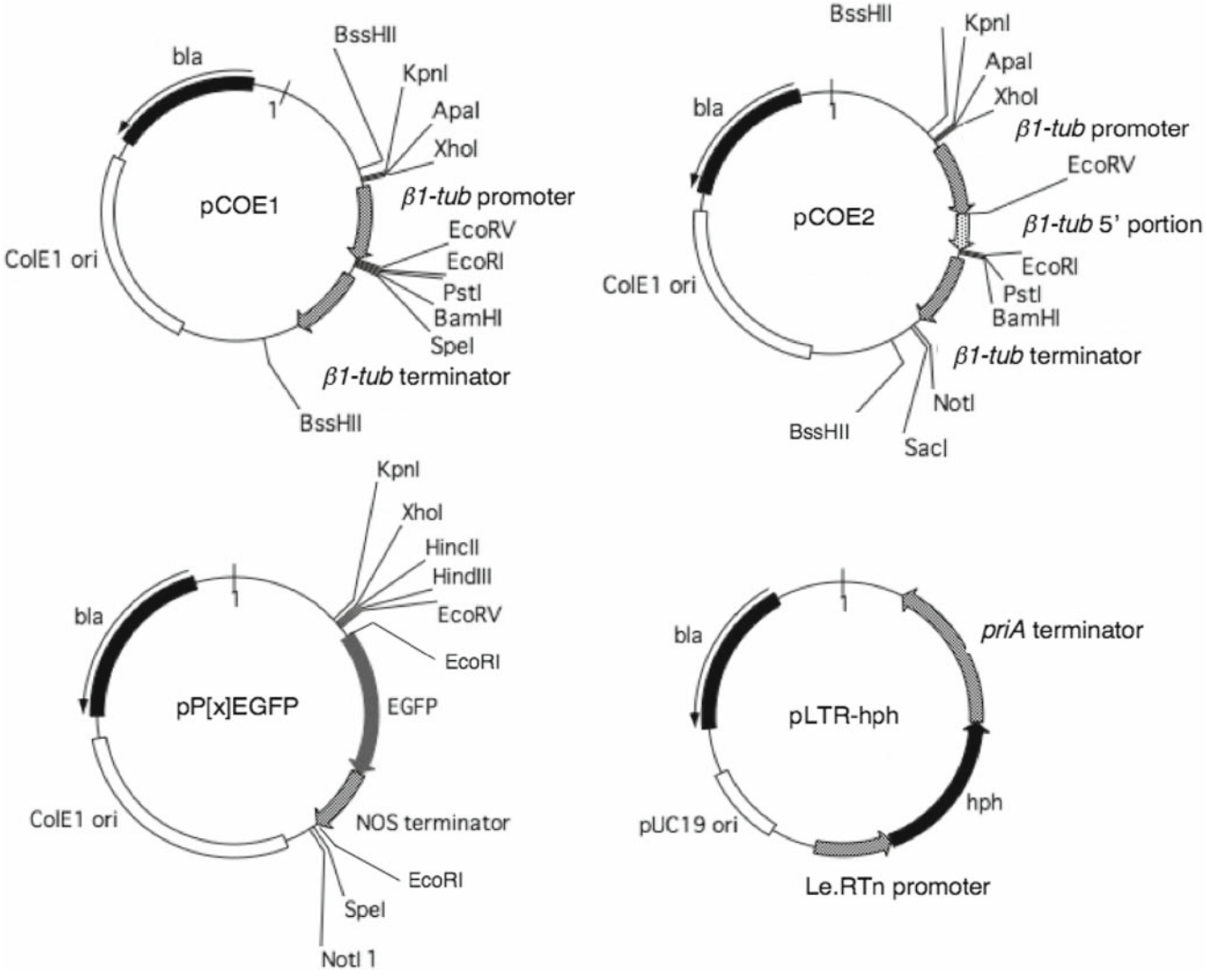

Fig. 1. Schematic representation of pCOE1, pCOE2, pP[x]EGFP, and pLTR-hph. The [x] in pP[x]EGFP indicates that the plasmid lacks the promoter and structural gene upstream of EGFP (efficient green fluorescent protein). For more details, see text

tance gene): pLC1-hph (Sato et al. 1998) was digested with *Sal*I and *Bam*HI to remove the *L. edodes ras* (*Le.ras*) promoter region (2.5 kb). The 474-bp fragment containing the long terminal repeat LTR of *L. edodes* retrotransposon *Le.*RTn1 [accession no. AB291605 in Shishido et al. (2007)] was inserted into it.

The plasmids pCOE2-EGFP and pP[Cc.ctg1]-Cc.Ctg1-EGFP used for expression of EGFP and Cc.Ctg1-EGFP were constructed as follows. pCOE2-EGFP: the aforementioned *Eco*RI-digested fragment containing the *egfp* gene and the *Nos* terminator was inserted into the *Eco*RI site of pCOE2. pP[Cc.ctg1]–Cc.Ctg1-EGFP: the *Cc.ctg1* fragment [nt (-)957–1192], which contains the *Cc.ctg1* promoter and almost all coding sequences, was prepared by PCR using *Cc.ctg1* f5 and r5 primers (see Table 1) was digested with *Hin*dIII and *Eco*RV. The resulting fragment was inserted into the site between *Hin*dIII and *Eco*RV of pP[x]EGFP. Transformations of vegetative hyphal cells of *C. cinerea* #326 using the recombinant plasmids were done according to the method described by Cummings et al. (1999) using pLTR-hph instead of pPHT1.

Semiquantitative RT-PCR (qRT-PCR) analysis

Analysis of *Cc.cdc5*, *Cc.ctg1* and *Cc.cipB* was done according to the method of Nakazawa et al. (2008b). Total cellular RNA was isolated from vegetative mycelia cultured in MYG liquid medium, fruiting bodies at various developmental stages, and parts of mature fruiting bodies of *C. cinerea* strain #326 using an RNAqueous kit and Plant Aid (Ambion, Foster City, CA, USA). The same amount (1 μg each) of the RNA samples was subjected to reverse transcription with a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Austin, TX, USA). The synthesized single-stranded cDNAs (10 ng each) were subjected to conventional PCR (95°C, 30 s; 57°C, 30 s; 72°C, 10 s; cycle number was 25) using Blend Taq (Toyobo). The

sets of the primers used for the RT-PCR analysis were *Cc.cdc5* f3 and *Cc.cdc5* r3, *Cc.ctg1* f1 and *Cc.ctg1* r2, *Cc.cipB* f2 and *Cc.cipB* r2, and β*1-tub* qRT-PCR f and β*1-tub* qRT-PCR r (see Table 1).

Fluorescence microscopy

Fruiting-body tissues of *C. cinerea* were fixed and embedded in paraffin according to procedures reported previously (Nakazawa et al. 2006). Longitudinal 10-μm ultrathin cryosections were mounted onto slides coated with poly-L-lysine. All fluorescence images were collected with an Olympus BX60 system. The mirror unit U-MGFPHQ (bandpass excitation, 460–480 nm; longpass emission, 495–540 nm) (Olympus, Tokyo, Japan) was used for analysis of EGFP fluorescence.

Results

Identification of *L. edodes Le.cdc5*, *ctg1*, and *cipB* homologues in *C. cinerea* and structural analysis of the gene homologues and their deduced products

We searched the databases of *C. cinerea* Okayama 7 genome (Broad Institute: http://www.broad.mit.edu/annotation/genome/coprinus_cinereus/Home.html) and the EST (Expressed Sequence Tag) database of *C. cinerea* (Fungal EST annotation database: https://fungalgenomics.concordia.ca/feadb/search.php?sp_index=ledo) for *L. edodes Le.cdc5*, *ctg1*, and *cipB* homologues. A *Le.cdc5* homologue of *C. cinerea* (*Cc.cdc5*) (accession no. XP_001833557) and a *cipB* homologue (*Cc.cipB*) (accession no. XP_001834060) were predicted in the genome database, and a *ctg1* homologue (*Cc.ctg1*) was found in the EST database (CcinSEQ20822).

Next, we amplified the full-length cDNAs of *Cc.cdc5* and *Cc.ctg1* from *C. cinerea* homokaryotic fruiting strain #326 (Pukkila and Casselton 1991; the recipient of DNA transformation used in this study) by RT-PCR using *Cc.cdc5* f3 and *Cc.cdc5* r1 and *Cc.ctg1* f2 and *Cc.ctg1* r2 (see Table 1). Sequence analysis showed that the *Cc.cdc5* and *Cc.ctg1* cDNAs of #326 have sequences identical to those of Okayama 7. We amplified full-length *Cc.cipB* cDNA from #326 by *Cc.cipB* f2 and *Cc.cipB* r3 (see Table 1) and compared with that of Okayama 7. There existed only a slight difference between #326 and Okayama 7: 135C (of Okayama 7) was replaced by T in #326, 184C by T, 225C by T, and 277G by A, resulting in generation of a single aa mutation, 93Tyr to Ala. The sequence data of *Cc.cdc5*, *Cc.ctg1* and *Cc.cipB* cDNAs of strain #326 can be found in the DDBJ/EMBL/GenBank nt sequence databases under the accession nos. AB465734, AB465735, and AB465736, respectively.

The primary structure of Le.CDC5 was compared with that of Cc.Cdc5. The N-terminal 104-aa sequence (c-Myb-type DNA-binding domain) of Le.CDC5 (842 aa residues; accession no. BAB62527) is completely identical to that of Cc.Cdc5 (822 aa residues), and Cc.Cdc5 also contain a leucine zipper in its C-terminal region. We also compared the aa sequences of *L. edodes* CTG1 (159 aa residues; accession no. BAF76763) and *C. cinerea* Ctg1 proteins (197 aa residues). As pictured in Fig. 2, both sequences showed significant similarity except for their C-terminal regions. As mentioned in the Introduction, CTG1 contains a short leucine zipper-like motif, AX_6LX_6L, at its N-terminus, and a short basic aa sequence, KKK, close to the C-terminus; Cc.Ctg1 contained the motif VX_6LX_6V at its N-terminus and the short basic aa sequence KRX_3RR close to its C-terminus.

Binding of Cc.Cdc5 and its interacting partner Cc.CipB to the *Cc.ctg1* gene in similar manner to the counterparts of *L. edodes*

Our previous study showed that the His (histidine)-tagged Le.CDC5 (aa 588–842) peptide containing a leucine zipper motif (the prey) formed a heterodimer with the GST-CIPB (aa 1–127) peptide (the bait) and a homodimer with the Le.CDC5 (aa 562–842) peptide (the bait) (Nakazawa et al. 2008a). In this report, we performed GST-pulldown analysis to study the interaction of Cc.Cdc5 with Cc.CipB (and Cc.Cdc5). The GST fusion proteins GST-Cc.Cdc5 (aa 568–822) with a leucine zipper motif and GST-Cc.CipB (aa 1–126) were used as bait and the HA-tagged Cc.Cdc5 (aa 568–822) peptide as prey. The results (Fig. 3) indicate that the HA (hemagglutinin)-tagged Cc.Cdc5 (aa 568–822) protein formed a complex with GST-Cc.CipB (aa 1–126) and a homodimer with GST-Cc.Cdc5 (aa 568–822).

Next, we studied the binding of Cc.Cdc5 and Cc.CipB to the *Cc.ctg1* gene. Our previous study showed that the Le.CDC5 (aa 1–185) peptide containing a c-Myb-type DNA-binding domain binds to a 7-bp sequence 5′-GCAATCT-3′(nt 322–328) in the transcribed region downstream of the

Fig. 2. Comparison of the amino acid (aa) sequences of *Lentinula edodes* CTG1 (159 aa residues) and *Coprinopsis cinerea* Ctg1 (Cc.Ctg1, 197 aa residues). The aa sequences were aligned to optimize matches using ClustalW. The aa residues identical to the CTG1 protein are *highlighted* (*black*) and similar aa residues are *shaded* (*gray*)

start codon of *ctg1* (Nakazawa et al. 2008a). As mentioned in the foregoing section, the N-terminal 104-aa sequence (c-Myb-type DNA-binding domain) of Cc.Cdc5 is completely identical to that of Le.CDC5, suggesting that Cc.Cdc5 recognizes/binds the sequences very similar to those of Le.CDC5. Computer search revealed that the *Cc.ctg1* gene (Contig 242: 120 820–122 052 in the *C. cinerea* genome database) contains a 7-bp sequence 5′-GCAAGCT-3′ (nt 583–589) in its transcribed region downstream of the start codon (Fig. 4A). To examine whether Cc.Cdc5 binds to the sequence 5′-GCAAGCT-3′, we performed electrophoretic mobility shift assay (EMSA) using a ^{32}P-labeled 169-bp fragment (nt 493–661) carrying it (probe 2 in Fig. 4A). As shown in lanes 8–12 in Fig. 4B, the Cc.Cdc5 (aa 1–200) peptide actually bound to probe 2. The shift band partially disappeared upon the addition of a 200-fold excess amount of the nonlabeled 169-bp fragment (competitor), suggesting a relatively tight binding of Cc.Cdc5 to the 7-bp sequence; in the case of Le.CDC5, the shift band almost disappeared upon the addition of a 100-fold excess amount of the competitor.

We previously reported that CIPB (127 aa residues) binds to a 6-bp sequence with the consensus sequence 5′-CAACAC/T/G-3′ (Nakazawa et al. 2008a). The 5′-upstream region [nt (-)149–2] of the *ctg1* gene contains six consensus-

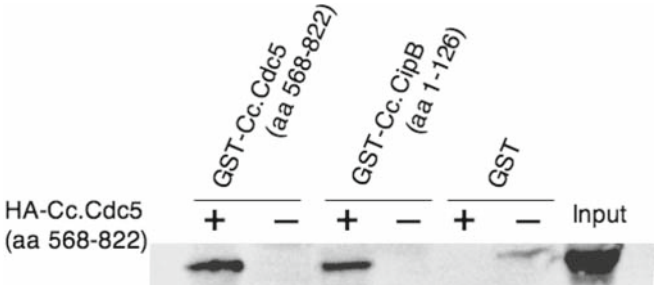

Fig. 3. Interaction of Cc.Cdc5 with Cc.CipB analyzed by glutathione *S*-transferase (GST)-pulldown analysis. The GST fusion (bait) proteins GST-Cc.Cdc5 (aa 568–822) and GST-Cc.CipB (aa 1–126) and GST per se bound to glutathione-Sepharose beads were exposed to a binding reaction with the supernatant of cell lysate containing the HA-tagged Cc.Cdc5 (aa 568–822) (prey) peptide and then immunoblotted. *Input:* The supernatant of cell lysate containing the HA-tagged Cc.Cdc5 (aa 568–822) peptide was directly electrophoresed and immunoblotted

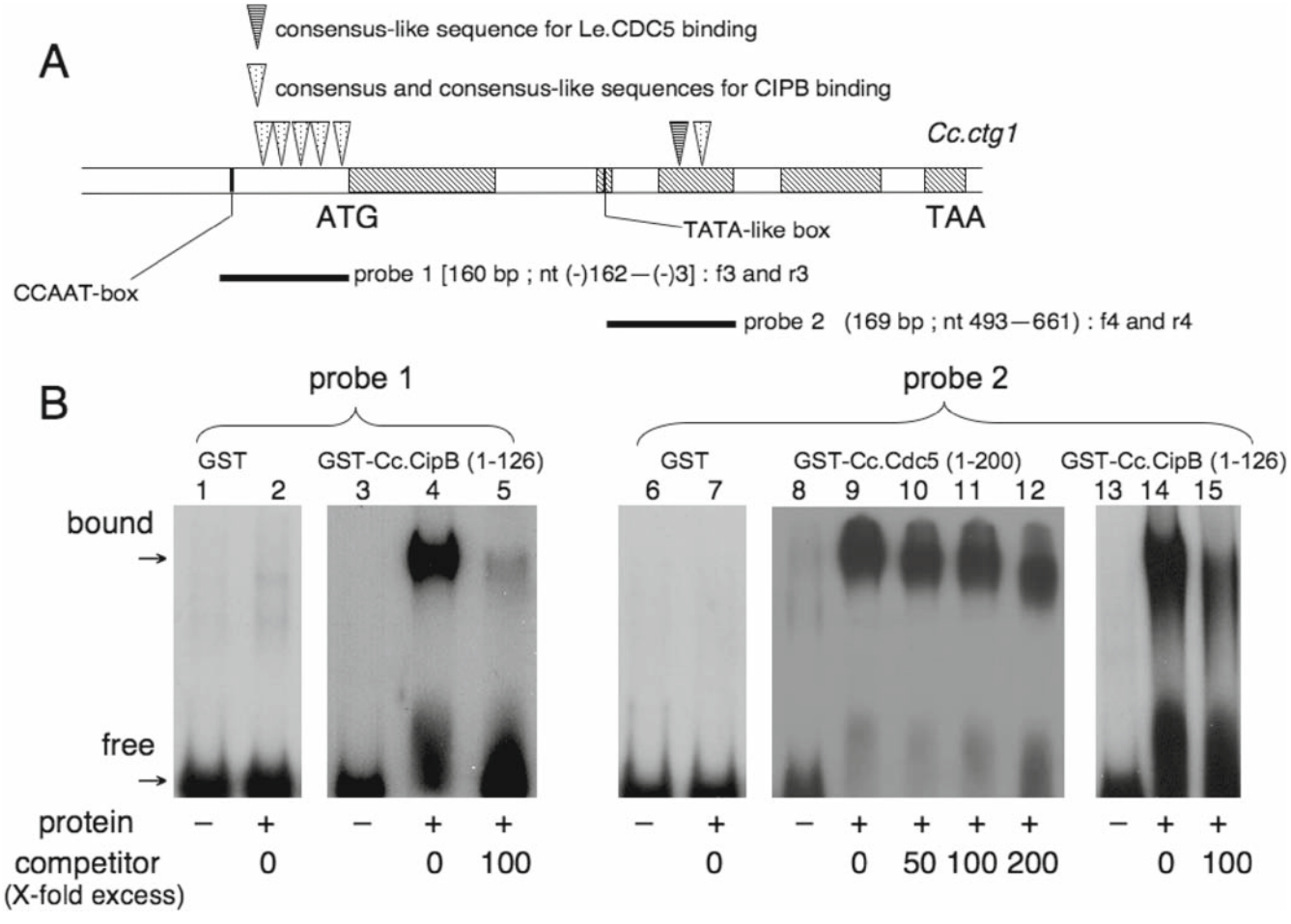

Fig. 4. Electrophoretic mobility shift analysis (EMSA) with the two ^{32}P-labeled fragments (probes 1 and 2) of the *Cc.ctg1* gene for binding to GST-Cc.Cdc5 (aa 1–200) and GST-Cc.CipB (aa 1–126) proteins. **A** Schematic representation of the *Cc.ctg1* gene and arrangement of the two fragments (probes) within the gene. **B** EMSA of probes 1 and 2 for binding to GST, GST-Cc.Cdc5 (aa 1–200), and GST-Cc.CipB (aa 1–126). *Lanes 1–5* contain 10 ng ^{32}P-labeled probe; *lanes 6–15* contain 10 ng ^{32}P-labeled probe 2. *Lanes 1*, *3*, *6*, *8*, *13*, none; *lanes 2*, *7*, GST (500 ng); *lanes 4*, *14*, GST-Cc.CipB (aa 1–126) (400 ng); *lane 5*, GST-Cc.CipB (aa 1–126) (400 ng) and 1000 ng nonlabeled probe 1; *lane 9*, GST-Cc.Cdc5 (aa 1–200) (400 ng); *lanes 10–12*, GST-Cc.Cdc5 (aa 1–200) (400 ng), and 500 ng (for *lane 10*), 1000 ng (for *lane 11*), 2000 ng (for *lane 12*) of nonlabeled probe 2; *lane 15*, GST-Cc.CipB (aa 1–126) (400 ng) and 1000 ng nonlabeled probe 2

like sequences and the transcribed region downstream start codon contains three consensus sequences of CIPB binding (one sequence is complementary). The aa sequence of CIPB shows 60.3% identity with that of Cc.CipB (Nakazawa et al. 2008a), suggesting that both proteins may bind similar 6-bp sequences. We searched the 5′-upstream and transcribed regions of the *Cc.ctg1* gene for the presence of the sequence(s) similar to a 6-bp CIPB-binding consensus (or consensus-like) sequence. The 5′-upstream region was found to contain five consensus and consensus-like sequences [5′-CAACcT-3′ (nt (-)106–(-)101), 5′-CAACtT-3′ (nt (-)75–(-)70), 5′-CAACcG-3′ (nt (-)58–(-)53), 5′-CAACAG-3′ (nt (-)52–(-)47), and 5′-CAAgAG-3′ (nt (-)37–(-)32); the small letters are the bases nonidentical with those of the consensus sequence of CIPB]. Also, the transcribed region downstream of the start codon exhibited a consensus-like sequence 5′-CAAgAC-3′ (nt 605–610). EMSA was performed using the ^{32}P-labeled 169-bp fragment (probe 2) described above and the 160-bp fragment [nt (-)162–(-)3] (probe 1 in Fig. 4A). As shown in lanes 3–5 and 13–15 in Fig. 4B, Cc.CipB bound to both probes. The shift bands of probe 1 and probe 2 almost or considerably disappeared upon the addition of 100-fold excess amounts of the nonlabeled fragments as competitors. These results suggest that Cc.CipB can bind to at least one of the five 6-bp consensus or consensus-like sequences in the 5′-upstream region and to a consensus-like sequence in the transcribed region. The results and previous observations, taken together, suggest that *Cc.ctg1* (in *C. cinerea*) and *ctg1* (in *L. edodes*) transcriptions may be regulated by a similar system.

Developmental regulation of the *Cc.cdc5*, *Cc.ctg1* and *Cc.cipB* expressions in *C. cinerea*

To investigate *Cc.cdc5*, *Cc.ctg1*, and *Cc,cipB* expressions in the course of fruiting-body formation and in parts of fruiting bodies of *C. cinerea* #326, we performed semiquantitative RT-PCR analysis (Fig. 5A) for the total cellular RNA

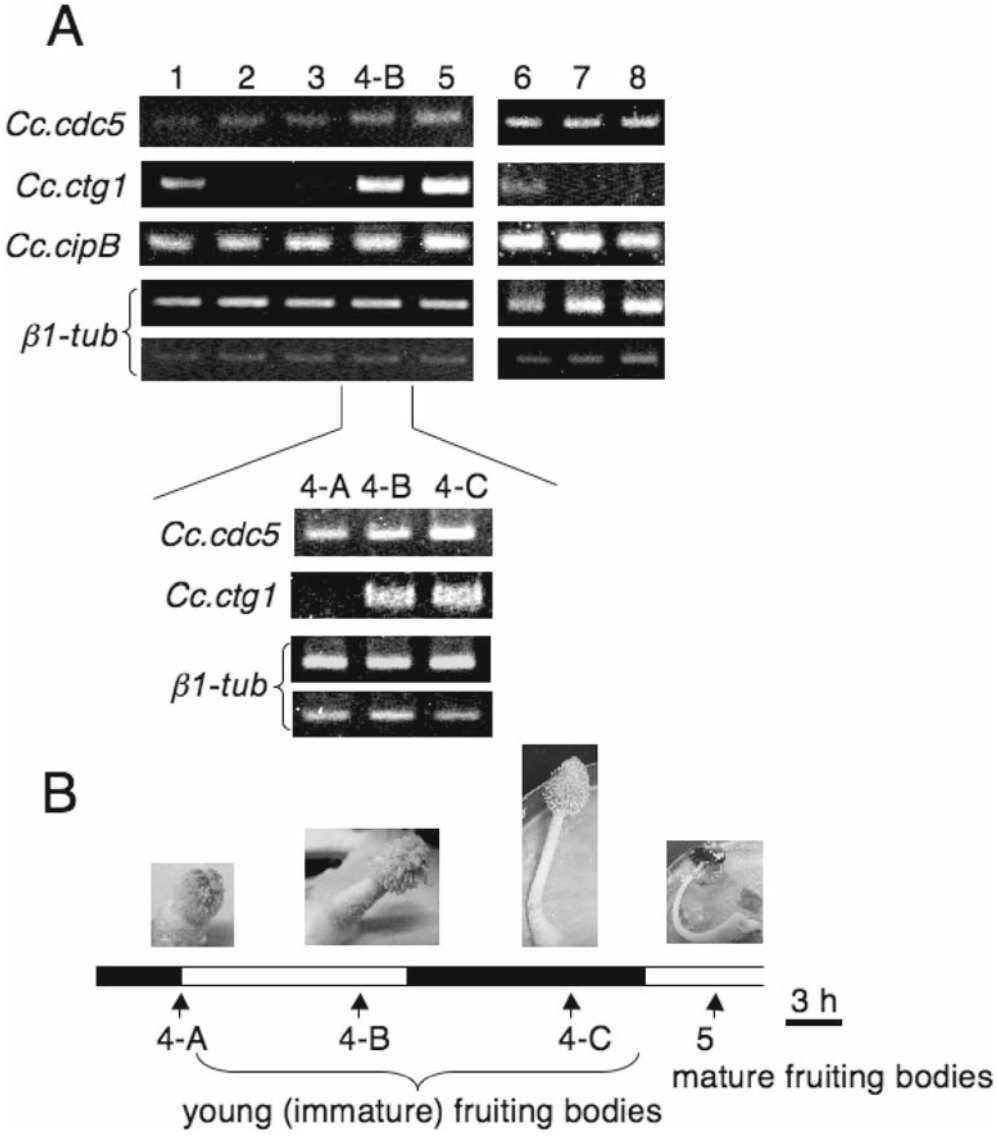

Fig. 5. Semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) of the transcripts of *Cc.cdc5*, *Cc.ctg1*, and *Cc.cipB* genes of *Coprinopsis cinerea* #326. **A** The same amount (1 μg each) of total cellular RNA was used for synthesis of single-stranded cDNAs. The RNA samples were isolated from vegetative hyphal cells (*lane 1*), fruiting-body initials (*lane 2*), stage 2 primordia (*lane 3*), stages 1–3 young (immature) fruiting bodies (*lanes 4-A–4-C*), mature fruiting bodies (*lane 5*), pileus (*lane 6*), stipe (*lane 7*), and primordial shaft (*lane 8*) of mature fruiting bodies. The synthesized single-stranded cDNAs (10 ng each) were subjected to conventional PCR. For the β*1-tub* gene, 10 and 1 ng synthesized single-stranded cDNAs were used. **B** The stages 1–3 young (immature) fruiting bodies (*4-A–4-C*) and mature fruiting bodies (*5*). Each of the stages (1–3) is indicated by a *vertical arrow*, together with the *horizontal bar* showing the cultivation under 12 h white light/12 h dark regime. The cycle number of the RT-PCR reactions was 25

samples prepared from vegetative hyphal cells (lane 1), fruiting-body initials (lane 2), stage 2 primordia (lane 3), stages 1–3 young (immature) fruiting bodies (lanes 4-A–4-C) and mature fruiting bodies (lane 5) (see Fig. 5B); these are defined according to Kues (2000). As shown in Fig. 5, although all the RNA samples contained the *Cc.cdc5* transcripts, stages 1–3 young fruiting bodies and mature fruiting bodies contained relatively large amounts of *Cc.cdc5* transcripts. Vegetative hyphal cells contained a small amount of the transcript. In the case of the *Cc.ctg1* transcript, stages 2 and 3 young fruiting bodies (lanes 4-B, 4-C) and mature fruiting bodies contained considerable amounts but stage 1 young fruiting bodies did not. Fruiting-body initials and stage 2 primordia also did not contain the transcript, but vegetative hyphal cells contained a considerable amount. Next, RT-PCR was done for the total cellular RNA samples prepared from pilei (lane 6), stipes (lane 7) and primordial shafts (lane 8) of mature fruiting bodies (see Fig. 5A). All the parts of mature fruiting bodies contained similar amounts of the *Cc.cdc5* transcript. On the other hand, *Cc.ctg1* transcript was contained exclusively in pilei. Differing from *Cc.cdc5* and *Cc.ctg1*, the transcript level of *Cc.cipB* was almost the same in the course of fruiting-body formation and in parts of fruiting bodies (see Fig. 5A). The transcript of β*1-tub*, which exhibits an almost constant level of expression, was analyzed as reference. We discuss later the lack of coincidence in the transcripts between *Cc.cdc5* and *Cc.ctg1*.

To investigate the distribution of the *Cc.ctg1* product (Cc.Ctg1) in pilei of stage 3 fruiting bodies (stage 4-C in Fig. 5B), we constructed the two chromosome-integrating plasmids, pP[Cc.ctg1]-Cc.Ctg1-EGFP (for production of Cc. Ctg1-EGFP fusion protein) and pCOE2-EGFP (for production of EGFP) (Fig. 6A), basically according to previous methods (Burns et al. 2005; Murata et al. 2006). These plasmids were introduced into *C. cinerea* #326, obtaining six transformants via pP[Cc.ctg1]-Cc.Ctg1-EGFP and three transformants via pCOE2-EGFP. We prepared longitudinal ultrathin cryosections from their pilei, obtaining good cryosections from the hymenophores (gill tissues). Because the pileus trama is a tissue with a low cell density, we obtained only sections of small mycelial pieces. A fluorescence microscopic analysis of the cryosections of hymenophores prepared from the six transformants revealed that the EGFP-fused Cc.Ctg1 protein is present exclusively in the hymenium. EGFP per se expressed by pCOE2-EGFP is present everywhere in the hymenophores from the three transformants. Results of a randomly selected transformant are shown in Fig. 6B.

Involvement of the *Cc.ctg1* gene (and the *ctg1* gene) in regulation of stipe elongation in *C. cinerea*

To study the biological function of the *Cc.ctg1* gene in *C. cinerea* #326 (a homokaryotic fruiting strain), we first attempted to obtain the gene-silenced vegetative hyphal transformants by the RNAi (RNA interference) method according to the method previously reported (Namekawa et al. 2005; Walti et al. 2006). For all our efforts, no transformant was obtained. A possible reason for this might be that the *Cc.ctg1* is involved in (or essential for) vegetative hyphal growth because the vegetative hyphal cells of #326 contain a considerable amount of *Cc.ctg1* transcript (see Fig. 5A). Thus, we attempted to obtain the vegetative hyphal transformant(s) producing a large amount of the *Cc.ctg1* transcript. A chromosome-integrating plasmid pCOE1-Cc.ctg1 (Figs. 1, 7A) was introduced into the *C. cinerea* #326 genome by cotransforming with pLTR-hph carrying the hygromycin B resistance gene (*hph*) (see Fig. 1) and selecting for Hyg^r transformants. A total of 20 Hyg^r clones were obtained and were screened for the presence of the *Cc.ctg1* expression cassette on their chromosomes by PCR. As *C. cinerea* carries the *Cc.ctg1* gene on the chromosome, we used the 5′-part of β*1-tub* terminator as forward primer (β*1-tub* terminator f1 primer in Table 1) and the M13 sequence downstream of β*1-tub* terminator (M13 r in Table 1) for reverse primer. Four Hyg^r transformants were selected by second screening and their total RNAs were analyzed by RT-PCR using *Cc.ctg1* f1 and *Cc.ctg1* r1 (Table 1). As shown in the upper part of Fig. 7B, all the four transformants, named TF-1[Cc.ctg1], TF-2[Cc.ctg1], TF-3[Cc. ctg1], and TF-4[Cc.ctg1], contained clearly larger amounts of *Cc.ctg1* transcripts than the recipient #326. Only TF-3[Cc. ctg1], however, did not form a fruiting body; probably a disruption of gene(s) essential for fruiting occurred by random integration of pCOE1-Cc.ctg1 and/or pLTR-hph on the chromosome(s). We investigated whether TF-1[Cc. ctg1], TF-2[Cc.ctg1], and TF-4[Cc.ctg1] show a different characteristic from #326 during the course of fruiting-body formation (the transformants and #326 were cultured under a 12-h white light/12-h dark regime). However, the transformants did not show any characteristic phenotype; the transformants and #326 exhibited simultaneous fruiting development (data not shown). Next, we investigated the phenotypes of the three transformants by a different way: stage 1 young (immature) fruiting bodies (Kues 2000), mainly consisting of developing pilei (caps) (4-A in Fig. 5B) were formed in the midnight–early morning hours through a 12-h white light/12-h dark regime and were then cultivated for 2 h in light condition (in the early morning). At this time (0 h in Fig. 8A), the three transformants and the recipient #326 showed very similar lengths of stipes (data not shown). We measured their lengths of stipes during further cultivation in the dark. At the 2-h and 4-h cultivations, the three transformants exhibited a rapid elongation of stipe compared with #326 (Fig. 8B). This characteristic phenotype of the transformants was confirmed by ten independent experiments. In Fig. 8, the relative lengths of stipes are shown (as relative stipe elongation) by the length of 0-h cultivation being taken as 1.0.

The same experiment was carried out for *L. edodes ctg1* by using a chromosome-integrating plasmid pCOE2-ctg1 (see Fig. 7A). Nine Hyg^r transformants obtained were analyzed by RT-PCR using pCOE2 qRT-PCR f and *ctg1* r (see Table 1). Four transformants, named TF-1[ctg1], TF-2[ctg1], TF-3[ctg1], and TF-4[ctg1], contained larger amounts of *ctg1* transcripts (bottom part, Fig. 7B). TF-1[ctg1], however,

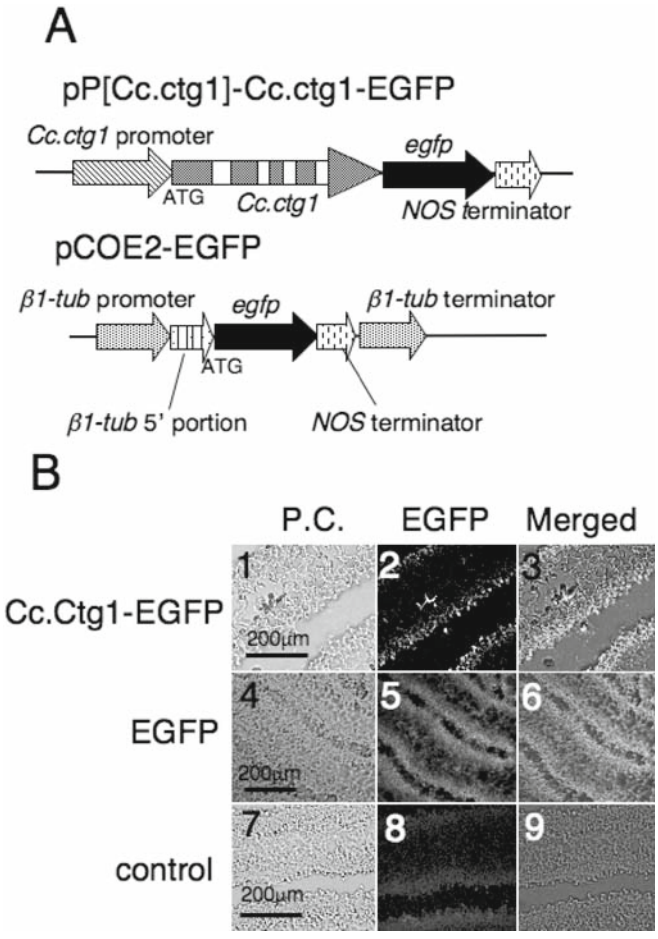

Fig. 6. Analysis of the distribution of the *Cc.ctg1* product (Cc.Ctg1) in the pilei (hymenophores) of stage 3 fruiting bodies of *Coprinopsis cinerea* #326. **A** Constructs of p[Cc.ctg1]-Cc.ctg1-EGFP and pCOE2-EGFP for production of EGFP or Cc.Ctg1-EGFP fusion protein. **B** Longitudinal 10-μm ultrathin cryosections of the pilei were examined microscopically using 40× objective. *Panels 1*, *4*, *7*, phase contrast; *2*, *5*, *8*, EGFP fluorescence; *3*, *6*, *9*, merged image. Cc.Ctg1-EGFP (*panels 1–3*) indicates ultrathin cryosections of the pilei of the transformant via p[Cc.ctg1]-Cc.ctg1-EGFP. EGFP (*panels 4–6*) indicates ultrathin cryosections of the pilei of the transformant via pCOE2-EGFP. Control (*panels 7–9*) indicates ultrathin cryosections of the pilei of the recipient #326

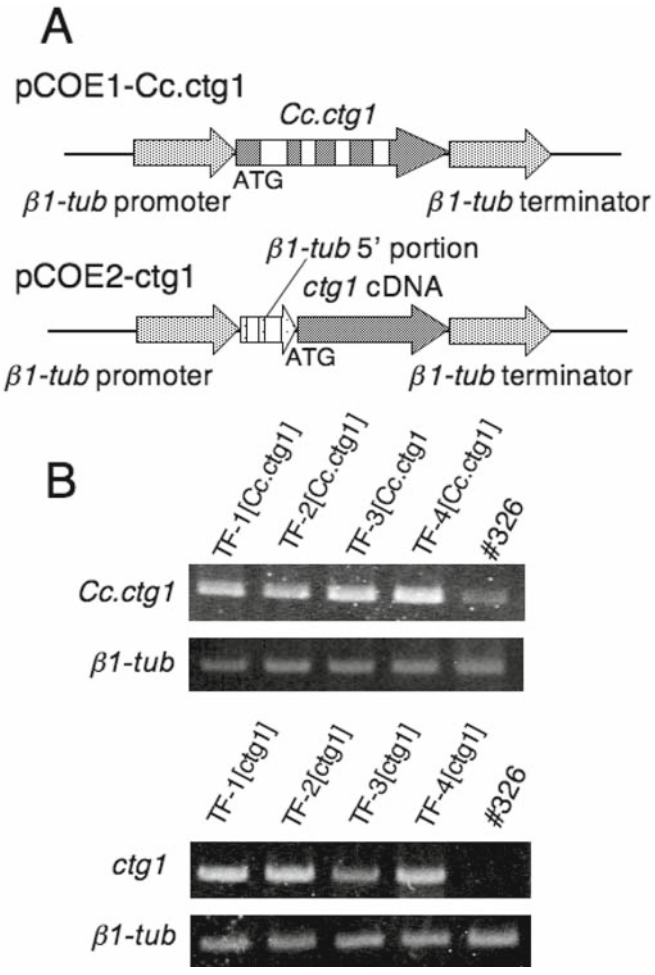

Fig. 7. Effect of overexpression of *Cc.ctg1* and *ctg1* on stipe elongation of *Coprinopsis cinerea*. **A** Constructions of pCOE1-Cc.ctg1 and pCOE2-ctg1. **B** Transcriptional expressions of *Cc.ctg1* and *ctg1* in vegetative hyphal cells of the transformants TF-1–4[Cc.ctg1] and TF-1–4[ctg1] and the recipient #326. The RT-PCR product was fractionated by gel electrophoresis in agarose gels and stained with ethidium bromide. The cycle number of the reaction was 25 for *Cc.ctg1* and 35 for *ctg1*. For more details, see Materials and methods

did not form a fruiting body. It is probable that the aforementioned gene disruption may occur. As shown in Fig. 8C, TF-2[ctg1], TF-3[ctg1], and TF-4[ctg1] also showed a rapid stipe elongation. The results strongly suggest that *Cc.ctg1* (*ctg1*) are involved in the regulation of stipe elongation in the fruiting maturation of *C. cinerea*. To support this, we carried out the following experiment. The *Coprinopsis cinerea dst1* mutant strain has been reported to form dark stipes under a 12-h light/12-h dark regime as well as under continuous darkness. *Coprinopsis cinerea* #326 also forms dark stipes under continuous darkness (Kues 2000; Terashima et al. 2005). The dark stipe mainly consists of an elongated primordial shaft; it possesses a rudimentary pileus and stipe attached on its top (Terashima et al. 2005). We investigated the transcript levels of *Cc.cdc5* and *Cc.ctg1* in dark stipes derived from #326 and its *dst1–4* mutant (Bm1) and found that these were negligibly low (data not shown).

Discussion

We demonstrated that *C. cinerea* Cdc5 (Cc.Cdc5), a homologue of Le.CDC5, binds to a 7-bp sequence 5′-GCAAGCT-3′ in the transcribed region downstream of the start codon of the *C. cinerea ctg1* gene (*Cc.ctg1*). *Coprinopsis cinerea* CipB, an interacting partner of Cc.Cdc5, binds to at least one of five 6-bp sequences in the 5′-upstream region and to a 6-bp sequence in the transcribed region downstream start codon of *Cc.ctg1*. These results implied that, similar to *L. edodes* (Nakazawa et al. 2008a), the gene expression regula-

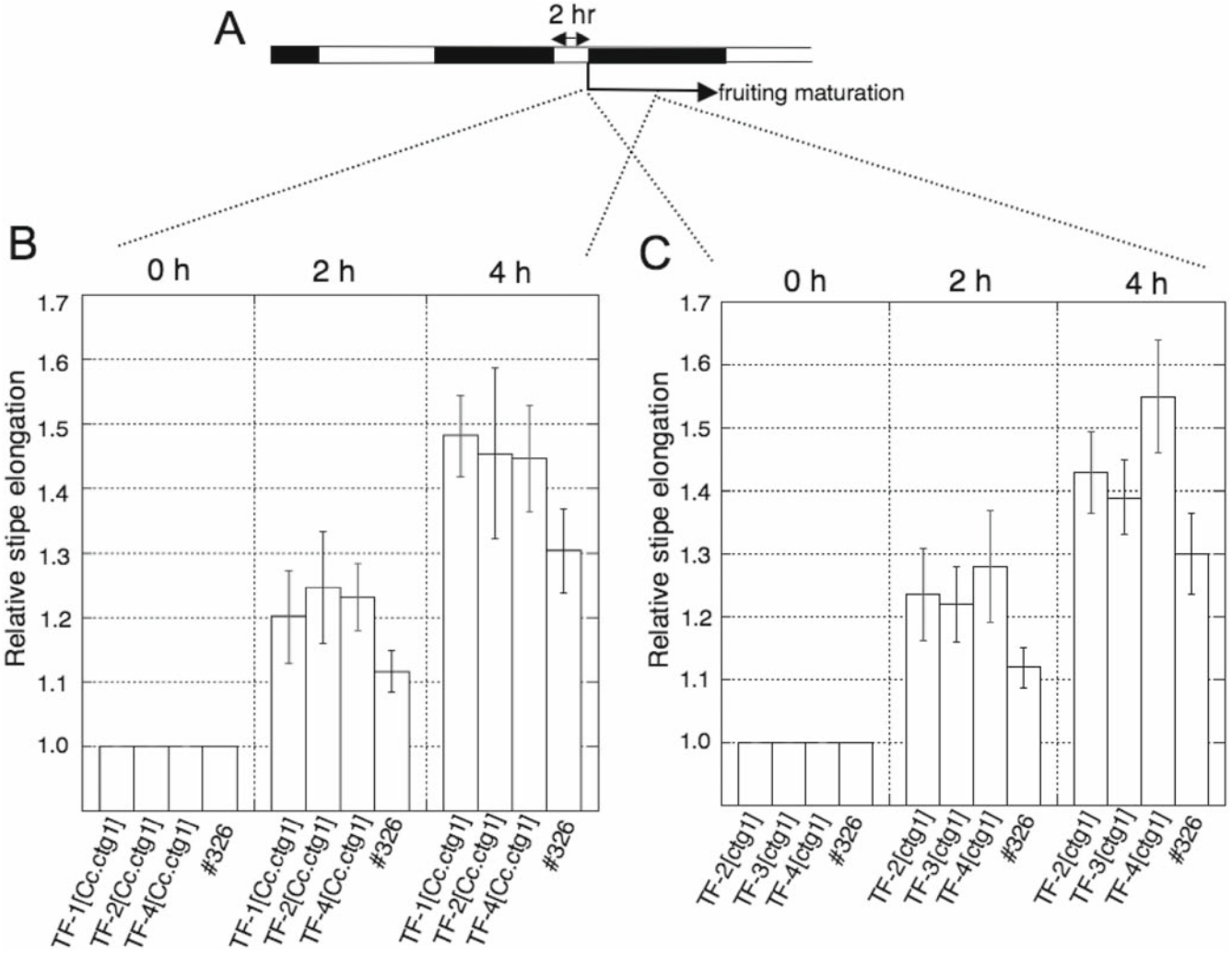

Fig. 8. Comparison of stipe elongation between the transformants with the recipient #326. **A** The stage 1 young (immature) fruiting bodies (refer to Fig. 6B), which were formed through a 12 h white light/12 h dark regime, were cultivated for 2 h in light condition and then cultivated in the dark. **B**, **C** Comparison of stipe elongation of TF-1[Cc.ctg1], TF-2[Cc.ctg1], and TF-4[Cc.ctg1] with the recipient #326 (**B**) and TF-2[ctg1], TF-3[ctg1], and TF-4[ctg1] (**C**) with #326. The lengths of the stipes were measured at the 0-h, 2-h, and 4-h cultivation times in the 12-h dark phase. Relative length of stipe (*vertical line*) is shown by the length of 0-h cultivation being taken as 1.0. *Error bars*, SD of ten replicates. For more details, see text

tion of *Cc.ctg1* by a cooperative binding of Cc.Cdc5 and Cc.CipB may occur in *C. cinerea*. RT-PCR analysis of *Cc.ctg1*, *Cc.cdc5*, and *Cc.cipB* transcripts in the course of fruiting-body formation and in parts of fruiting bodies suggests that *Cc.ctg1* transcription is regulated cooperatively by Cc.Cdc5 and Cc.CipB. The vegetative hyphal cells contained a noticeable amount of *Cc.ctg1* transcript, but they contained only a small amount of *Cc.cdc5* transcript (Fig. 5A). This finding implies the possibility that other transcription factor(s) also participate in *Cc.ctg1* transcription. Also, *Cc.ctg1* may be involved in vegetative hyphal growth. The fruiting-body initials, stage 2 primordia, stage 1 young (immature) fruiting bodies, and stipes and primordial shafts of mature fruiting bodies contained the *Cc.cdc5* transcript but they did not contain the *Cc.ctg1* transcript. These results suggest the presence of other target gene(s) for the Cc.Cdc5 transcription factor.

Concerning the biological function of the *Cc.ctg1* gene (and *ctg1*), the followings are considered and discussed. The *C. cinerea* homokaryotic fruiting strains producing larger amounts of the *Cc.ctg1* (or *ctg1*) transcript were shown to elongate their stipes more rapidly than the parental strain #326 (see Fig. 8A,B). At the time that stipe elongation just started under the irregular regime (not 12-h light/12-h dark), the expression of *Cc.ctg1* (or *ctg1*) occurred in the strains bred by the molecular genetic approach whereas it did not occur in #326 (refer to 4-A in Fig. 5A, B). Expression of *Cc.ctg1* and *Cc.cdc5* barely occurs in dark stipes, which mainly consists of an elongated primordial shaft to the top of which a rudimentary pileus and unelongated stipe are attached (Kues 2000; Terashima et al. 2005). These results seem likely to support our proposal described above, suggesting that *Cc.ctg1* (and *ctg1*) may play a role in the regulation of stipe elongation in *C. cinerea*. We have previ-

ously described that *L. edodes* CTG1 might be involved in inhibition of stipe elongation in *L. edodes*, judging from the observation that *ctg1* is expressed in mature fruiting bodies and repressed in small young fruiting bodies; stipes are fully elongated in mature fruiting bodies (Nakazawa et al. 2008a). The most recent detailed analysis showed that the amount of the *ctg1* transcript gradually increases in proportion to enlargement of the fruiting body (unpublished observations). These findings suggest that *ctg1* may be also involved in the regulation of stipe elongation and fruiting maturation in *L. edodes*. However, it was unexpected that the expression of *Cc.ctg1* in *C. cinerea* occurs exclusively in the pileus (cap), especially in its hymenium (gill tissues); no *Cc.ctg1* transcript is present in the stipe. This discrepancy led us to propose that stipe elongation is connected to cap development in *C. cinerea*; the *Cc.ctg1* product Cc.Ctg1 regulates cap development, which leads to stipe elongation. Indeed, Kamada (1994) reported that stipe elongation depends on the presence of cap (pileus) in *C. cinerea*. The correlation of pileus (gills) and stipe elongation had been reported in other edible basidiomycetous mushrooms *Flammulina velutipes* and *Agaricus bisporus* (Gruen 1963, 1969, 1982).

The *eln* genes essential for stipe elongation have been identified in *C. cinerea* (Muraguchi and Kamada 2000; Arima et al. 2004). The *eln2* gene encodes a novel type of cytochrome P450 enzyme (CYP502) and is constitutively expressed (Muraguchi and Kamada 2000). So far, cytochrome P450 enzymes have been reported to be involved in producing phytohormones in plants (Winkier and Helentjaris 1995; Feldmann 2001). Furthermore, a variety of developmentally specific genes encoding several types of cytochrome P450 enzymes and several proteins involved in membrane transport and endocytosis have been identified in edible mushrooms *L. edodes*, *Pleurotus ostreatus*, and *F. velutipes* (Akiyama et al. 2002; Miyazaki et al. 2005; Yamada et al. 2006; Joong-Ho et al. 2007; Lee et al. 2007). These results led us to propose another possibility: both *Cc.ctg1* (Cc.Ctg1) and *ctg1* (CTG1) are involved in production (or regulation of production) or transport of a sort of signal compound. Kamada and Tsuji (1979) reported that signal compound(s) produced in the pileus by dark treatment are essential for the fruiting maturation (stipe elongation and pileus expansion/autolysis) in *Coprinus macrorhizus*, a synonym of *C. cinerea*. In contrast to the *Cc.ctg1* transcript in *C. cinerea*, the *ctg1* transcript is present mostly in the upper halves of the stipes and also in the hymenophore-depleted pileus of *L. edodes*. *Lentinula edodes* and *C. cinerea* display different tissue organization patterns in the development and maturation of their fruiting bodies (Moore 1998). In *L. edodes*, the signal compound produced in the pileus moves into an upper part of stipe and that produced in the stipe remains there. In either possibility, the molecular mechanism of the gene function of *Cc.ctg1* in *C. cinerea* (and *ctg1* in *L. edodes*) remains to be studied.

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and Research Fellowships of the Japan Society for the Promoting of Science (JSPS) for Young Scientists.

References

Ajuh P, Kuster B, Panov K, Zomerdijk JC, Mann M, Lamond AI (2000) Functional analysis of the human CDC5L complex and identification of its components by mass spectrometry. EMBO J 19: 6569–6581

Akiyama R, Sato Y, Kajiwara S, Shishido K (2002) Cloning and expression of cytochrome P450 genes, belonging to a new P450 family, of the basidiomycete *Lentinula edodes*. Biosci Biotechnol Biochem 66: 2183–2188

Arima T, Yamamoto M, Hirata A, Kawano S, Kamada T (2004) The *eln3* gene involved in fruiting body morphogenesis of *Coprinous cinereus* encodes a putative membrane protein with a general glycosyltransferease domain. Fungal Genet Biol 41:805–812

Bernstein HS, Coughlin SR (1998) A mammalian homolog of fission yeast Cdc5 regulates G_2 progression and mitotic entry. J Biol Chem 273:4666–4671

Burns CG, Ohi R, Krainer AR, Gould KL (1999) Evidence that Myb-related CDC5 proteins are required for pre-mRNA splicing. Proc Natl Acad Sci U S A 96:13789–13794

Burns C, Gregory KE, Kirby M, Cheung MK, Riquelme M, Elliott TJ, Challen MP, Bailey A, Foster GD (2005) Efficient GFP expression in the mushrooms *Agaricus bisporus* and *Coprinus cinerea* requires intron. Fungal Genet Biol 42:191–199

Chum WWY, Ng KTP, Shih RSM, Au CH, Kwan HS (2008) Gene expression studies of the dikaryotic mycelium and primordium of *Lentinula edodes* by serial analysis of gene expression. Mycol Res 112:950–964

Cummings WJ, Celerin M, Crodian J, Brunick LK, Zolan ME (1999) Insertional mutagenesis in *Coprinus cinereus*: use of a dominant selectable marker to generate tagged, sporulation-defective mutants. Curr Genet 36:371–382

Feldmann KA (2001) Cytochrome P450s as genes for crop improvement. Curr Opin Plant Biol 4:162–167

Gruen HE (1963) Endogenous growth regulation in carpophore of *Agaricus bisporus*. Plant Physiol 38:652–666

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

Gruen HE (1982) Control of stipe elongation by the pileus and mycelium in fruit bodies of *Flammulina velutipes* and other agaricales. In: Wells K, Wells EK (eds) Basidium and basidiocarp evolution, cytology, function and development. Springer, New York, pp 125–155

Hirayama T, Shinozaki K (1996) A $cdc5^+$ homolog of a higher plant *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 93:13371–13376

Inoue S, Kondo S, Hashimoto M, Kondo T, Muramatsu M (1991) Isolation of estrogen receptor-binding sites in human genomic DNA. Nucleic Acids Res 19:4091–4096

Joong-Ho J, Seung-Ho L, Jin-Sung L, Ki-Hwan K, Sung-Ja J, Woong-Han Y, Nam-Kuk K, Eun-Suk S, Yong-Sup C, Young-Bok Y, Chang-Soo L, Beom Gi K (2007) Isolation of genes expressed during the developmental stages of the oyster mushroom, *Pleurotus ostreatus*, using expressed sequence tags. FEMS Microbiol Lett 276:19–25

Kamada T (1994) Stipe elongation in fruit bodies. In: Wessels JGH, Meinhardt F (eds) The Mycota, vol. I. Growth, differentiation and sexuality. Springer-Verlag, Berlin, pp 367–380

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

Kues U (2000) Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol Mol Biol Rev 64:316–353

Lee MT, Szeto CYY, Ng TP, Kwan HS (2007) Endocytosis in the shiitake mushroom *Lentinula edodes* and involvement of GTPase LeRAB7. Eukaryot Cell 6:2406–2418

Lei XH, Shen X, Xu XQ, Bernstein HS (2000) Human Cdc5, a regulator of mitotic entry, can act as a site-specific DNA binding protein. J Cell Sci 113:4523–4531

Leung GSW, Zhang M, Xie WJ, Kwan HS (2000) Identification by RNA fingerprinting of genes differentially expressed during the development of the basidiomycete *Lentinula edodes*. Mol Gen Genet 262:977–990

Lin Z, Yin K, Wang X, Liu M, Chen Z, Gu H, Qu L (2007) Virus induced gene silencing of *AtCDC5* results in accelerated cell death in *Arabidopsis* leaves. Plant Physiol Biochem 45:87–94

Lugones LG, Scholtmeijer K, Klootwijk R, Wessel JG (1999) Introns are necessary for mRNA accumulation in *Schizophyllum commune*. Mol Microbiol 32:681–689

Makino R, Kamada T (2004) Isolation and characterization of mutations that affect nuclear migration for dikaryosis in *Coprinus cinereus*. Curr Genet 45:149–156

McDonald WH, Ohi R, Smelkova N, Frendewey D, Gould KL (1999) Myb-related fission yeast cdc5p is a component of a 40 snRNP-containing complex and is essential for pre-mRNA splicing. Mol Cell Biol 19:5352–5362

Miyazaki Y, Jojima T, Ono T, Yamazaki T, Shishido K (2004) A cDNA homologue of *Schizosaccharomyces pombe cdc5*$^{+}$ from the mushroom *Lentinula edodes*: characterization of the cDNA and its expressed product. Biochim Biophys Acta 1680:93–102

Miyazaki Y, Nakamura M, Babasaki K (2005) Molecular cloning of developmentally specific genes by representational difference analysis during the fruiting body formation in the basidiomycete *Lentinula edodes*. Fungal Genet Biol 42:493–505

Moore D (1998) Fungal morphogenesis. Cambridge University Press, Cambridge, pp 246–392

Muraguchi H, Kamada T (2000) A mutation in the *eln2* gene encoding a cytochrome P450 of *Coprinus cinereus* affects mushroom morphogenesis. Fungal Genet Biol 29:49–59

Murata H, Sunagawa M, Yamazaki T, Shishido K, Igasaki T (2006) Expression of the autofluorescent protein, DsRed2, in the recombinants of the ectomycorrhizal basidiomycete, *Suillus grevillei*, generated by *Agrobacterium*-mediated transformation. Mycorrhiza 16:407–412

Nakazawa T, Miyazaki Y, Kaneko S, Shishido K (2006) Developmental regulator Le.CDC5 of the mushroom *Lentinula edodes*: analyses of its amount in each of the stages of fruiting-body formation and its distribution in parts of the fruiting bodies. FEMS Microbiol Lett 261:60–63

Nakazawa T, Kaneko S, Miyazaki Y, Jojima T, Yamazaki T, Katsukawa S, Shishido K (2008a) Basidiomycete *Lentinula edodes* CDC5 and a novel interacting protein CIPB bind to a newly isolated target gene in an unusual manner. Fungal Genet Biol 45:818–828

Nakazawa T, Miyazaki Y, Kaneko S, Shishido K (2008b) Stimulative effects of light and a temperature downshift on transcriptional expressions of developmentally regulated genes in the initial stages of fruiting-body formation of the basidiomycetous mushroom *Lentinula edodes*. FEMS Microbiol Lett 289:67–71

Namekawa HS, Iwabata K, Sugawara H, Hamada NF, 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 151:3669–3678

Ohi R, McCollum D, Hirani B, Den Haese GJ, Zhang X, Burke JD, Turner K, Gould KL (1994) The *Schizosaccharomyces pombe cdc5*$^{+}$ gene encodes an essential protein with homology to c-Myb. EMBO J 13:471–483

Pukkila PJ, Casselton LA (1991) Molecular genetics of the agaric *Coprinus cinereus*. In: Bennett JW, Lasure LL (eds) More gene manipulations in fungi. Academic Press, San Diego, pp 127–150

Sato T, Yaegashi K, Ishii S, Hirano T, Kajiwara S, Shishido K, Enei H (1998) Transformation of the edible basidiomycete *Lentinula edodes* by restriction enzyme-mediated integration of plasmid DNA. Biosci Biotechnol Biochem 62:2346–2350

Shishido K, Nobusaka R, Kaneko S, Akiyama R, Miyazaki Y, Ninomiya M, Katsukawa S (2007) Long terminal repeat of the mushroom retrotransposon functions as a dual terminator to transcription. Biosci Biotechnol Bochem 71:2321–2324

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

Tsai WY, Chow YT, Chen HR, Huang KT, Hong RI, Jan SP, Kuo NY, Tsao TY, Chen CH, Cheng SC (1999) Cef1p is a component of the Prp19-associated complex and essential for pre-mRNA splicing. J Biol Chem 274:9455–9462

Walti MA, Villalba C, Buser RM, Grunler A, Aebi M, Kunzler M (2006) Targeted gene silencing in the model mushroom *Coprinopsis cinerea* (*Coprinus cinereus*) by expression of homologous hairpin RNAs. Eukaryot Cell 5:732–744

Winkler RG, Helentjaris T (1995) The Maize *Dwarf3* gene encodes a cytochrome P450-mediated early step in gibberellin biosynthesis. Plant Cell 7:1307–1317

Yamada M, Sakuraba S, Shibata K, Taguchi G, Inamoto S, Okazaki M, Shimosaka M (2006) Isolation and analysis of genes specifically expressed during fruiting body development in the basidiomycete *Flammulina velutipes* by fluorescence differential display. FEMS Microbiol Lett 254:165–172

Yamazaki T, Okajima Y, Kawashima H, Tsukamoto A, Sugiura J, Shishido K (2006) Intron-dependent accumulation of mRNA in *Coriolus hirsutus* of lignin peroxidase gene the product of which is involved in conversion/degradation of polychlorinated aromatic hydrocarbons. Biosci Biotechnol Biochem 70:1293–1299