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Regular Articles

The *Coprinopsis cinerea* septin Cc.Cdc3 is involved in stipe cell elongation

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ABSTRACT

We have identified and characterized a *Coprinopsis cinerea* mutant defective in stipe elongation during fruiting body development. In the wild-type, stipe cells elongate at the maturation stage of fruiting, resulting in very slender cells. In the mutant, the stipe cells fail to elongate, but become rather globular at the maturation stage. We found that the mutant phenotype is rescued by a gene encoding a homolog of *Saccharomyces cerevisiae* CDC3 septin, Cc.Cdc3. The *C. cinerea* genome includes 6 septin genes, 5 of which, including *Cc.cdc3*, are highly transcribed during stipe elongation in the wild type. In the mutant, the level of *Cc.cdc3* transcription in the stipe cells remains the same as that in the mycelium, and the level of *Cc.cdc10* transcription is approximately 100 times lower than that in the wild-type stipe cells. No increase in transcription of *Cc.cdc3* in the mutant may be due to the fact that the *Cc.cdc3* gene has a 4-base pair insertion in its promoter and/or that the promoter region is methylated in the mutant. Overexpressed EGFP-Cc.Cdc3 fusion protein rescues the stipe elongation in the transformants, localizes to the cell cortex and assembles into abundant thin filaments in the elongating stipe cells. In contrast, in vegetative hyphae, EGFP-Cc.Cdc3 is localized to the hyphal tips of the apical cells of hyphae. Cellular defects in the mutant, combined with the localization of EGFP-Cc.Cdc3, suggest that septin filaments in the cell cortex provide the localized rigidity to the plasma membrane and allow cells to elongate cylindrically.

1. Introduction

The basidiomycete *Coprinopsis cinerea* forms a highly differentiated structure, the fruiting body (Muraguchi and Kamada; 1998; Kües, 2000). Fig. 1 is a schematic diagram illustrating fruiting in *C. cinerea*. Fruiting body formation begins with the aggregation of hyphae, producing hyphal knots of approximately 0.2 mm or less in diameter. In the hyphal knots, cells divide rapidly and differentiate into a compact core composed of highly branched short cells and a layer of veil cells covering the core (van der Valk and Marchant, 1978). Following differentiation of the primordial shaft, the rudimentary pileus (cap) differentiates at the upper region of the primordial shaft, forming a tiny fruiting body primordium (Muraguchi and Kamada, 1998). The stipe tissue differentiates at the central region encompassed by the rudimentary gill. The primordium gradually enlarges and matures under proper light conditions, such as a 12 h light/12 h dark cycle (Kamada et al., 1978; Terashima et al., 2005). The maturation stage is triggered by light (0 h in Fig. 1), and stipe elongation starts around the end of the light period in the final day (Kamada, 1994). During stipe elongation, stipe cells elongate without cell division and with nuclear division, becoming multinucleate (Gooday, 1985; Stephenson and Gooday, 1984). The pileus expands to disperse basidiospores near the end of stipe elongation. Pileus expansion and spore dispersion are associated with fruiting body autolysis (Muraguchi et al., 2008), which characterizes the ephemeral life of the *Coprinopsis* genus.

In fruiting, two types of cell expansion occur: a slow process, which is often encountered in primordia, and a more rapid one involved specifically in stipe elongation (Reijnders and Moore, 1985). Stipe elongation is almost entirely due to elongation of the stipe cells, which elongate from approximately 0.1 mm to 1 mm during the final phase of fruiting, providing a good opportunity to study cell expansion that is characterized by diffuse extension growth (Kamada and Takemaru, 1977; Kamada, 1994; Gooday, 1985). Diffuse extension growth, unlike tip growth in vegetative hyphae, occurs throughout the cell surface; the helical or transverse arrangement of chitin microfibrils or glucosaminoglycan chains in the cell wall contributes to the process (Gooday, 1979; Kamada et al., 1991; Mol et al., 1990). Such microfibril arrangement was observed in an initial stage of fruiting body development, i.e., in

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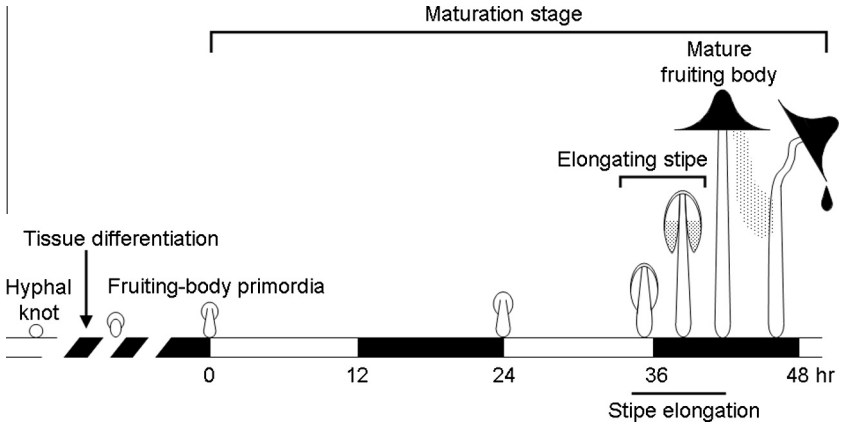

Fig. 1. Schematic diagram of *C. cinerea* fruiting. Tissue differentiation occurs in hyphal knots to produce fruiting-body primordia, which enlarge up to approximately 1 cm in height. The maturation stage of fruiting is triggered by light a day before the final day of fruiting. The 12 h light/12 h dark cycle is indicated by open and filled boxes, respectively, under the diagram. The time from the trigger light is shown in hr under the light regime, as previously described (Kamada et al., 1978).

hyphal knots of 0.1–0.2 mm in diameter (Kamada and Tsuru, 1993). Models have been proposed for the ontogeny and construction of the helical wall structure (Kamada et al., 1991). However, little is known about the molecular mechanisms underlying diffuse extension growth.

We have isolated *elongationless* mutants, which fail to elongate the stipe during the maturation stage of fruiting. Genetic analysis of the mutants identified at least eight genes, *eln1* to *eln8*, involved in stipe elongation. Of the eight genes, *eln2* and *eln3* were cloned and found to encode a cytochrome P450 and a putative glycosyltransferase, respectively (Muraguchi and Kamada, 2000; Arima et al., 2004). In this study, we investigated the *elongationless* mutant Uad435, which was induced in a homokaryotic fruiting strain, CopD5-12 (Muraguchi et al., 1999). In this report, we show that the gene responsible for the *elongationless* phenotype in Uad435 encodes a septin protein similar to the *Saccharomyces cerevisiae* CDC3, designated *Cc.cdc3*. Subcellular localization of Cc.Cdc3 was examined in stipe, veil, and vegetative hyphal cells using EGFP-tagged Cc.Cdc3. The results suggest that cortical septin filaments provide the localized rigidity to the plasma membrane of stipe cells and allow stipe cells to elongate cylindrically.

2. Materials and methods

2.1. Strains, culture conditions and genetic techniques

The strains of *C. cinerea* used in this study are listed in Table 1. Strain Uad435 carries the *eln8-1* recessive mutation, which was induced by UV-mutagenesis of homokaryotic fruiting strain CopD5-12 (Muraguchi et al., 1999). An *eln8-1* homozygous strain, B87+D365, was used to observe the mutant phenotype. Malt extract–yeast extract–glucose (MYG) medium (Rao and Niederpruem, 1969) solidified with 1.5% (w/v) agar was used for all experiments. MYG slant medium in test tubes was used to observe fruiting phenotypes. To obtain F_1 progeny, basidiospore germlings were isolated at random using a chisel-shaped needle under a dissecting microscope (Miles et al., 1966). The mycelium was cultured on CY-1 medium (Kamada and Takemaru, 1977) at 28 °C to observe hyphal cells.

2.2. Transformation experiments

To obtain a recipient strain for transformation experiments, the original mutant strain Uad435 (*A12 B12 eln8-1*) was crossed with #292 (*A3 B1 trp1-1,1-6*). Among the F_1 progeny, strain B87 (*A12 B1 trp1-1,1-6 eln8-1*) was selected as the recipient strain. Tester strain D365 (*Amut Bmut pab1-1 eln8-1*) was selected among F_1 progeny derived from a cross between Uad435 and #326 (*Amut Bmut pab1-1*).

Protoplasts of strain B87 were obtained from oidia and transformed with BAC DNAs or DNA fragments using a PEG-Ca^{2+} method as described previously (Binninger et al., 1987; Muraguchi et al., 2005). Trp^+ transformants were crossed with tester strain D365 in MYG slant medium to observe the fruiting phenotype.

BAC DNA of s14B9 was digested partially with *Hin*dIII and fractionated with CHEF electrophoresis. The gel portion containing fragments greater than 40 kb was excised and subjected to electroelution as described (Muraguchi et al., 2005). The recovered fragments were self-ligated and transformed into competent DH10B cells to construct a sub-library. DNA was extracted from subclones of s14B9 and examined for rescuing ability. Transformation experiments using subclones derived from s14B9 and end-sequencing of the subclones with the rescuing activity narrowed the active region to about 15 kb in the *C. cinerea* genome browser (http://genome.semo.edu/cgi-bin/gbrowse/cc/). Within the 15-kb region was the septin-coding region (Fig. S2). A septin gene containing approximately 1 kb of the 5′- and 3′-flanking regions was PCR-amplified, used for co-transformation with pCc1003 (Binninger et al., 1987), and found to carry the rescuing activity.

2.3. Phylogenetic analysis

Amino-acid sequences of fungal septins were first aligned using ClustalW (http://www.genome.jp/tools/clustalw/). Next, the aligned sequences were used to generate a phylogenetic tree by executing a command: rooted phylogenetic tree with branch length (UPGMA) in the ClustalW site. To distinguish used amino-acid sequences of septins from filamentous fungi, assigned numbers of the sequences and the number of amino acids were indicated in Fig. 3.

2.4. Expression analysis

Total RNA was extracted from the mycelium, fruit-body primordium, cap, and elongating stipe using RNAiso solution (TaKaRa Bio) and then used for northern blot analysis, quantitative real-time PCR, and super-SAGE. Fruit-body primordia were harvested at 0–12 h in Fig. 1. The stipe and cap tissues were harvested from the fruit bodies at around 35 h in Fig. 1.