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SHORT COMMUNICATION

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cAMP promotes hyphal branching in *Mucor globosus*

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Abstract The growth pattern of *Mucor globosus* cultured on a medium with or without cyclic adenosine monophosphate (cAMP) was examined. Branching remarkably increased in a mycelium grown on an agar medium containing cAMP. In submerged culture containing cAMP, some sporangiospores grew spherically and formed yeast-like cells, and others showed hyphal growth. These hyphae showed septation and swelling and formed spore-like structures. When these hyphae were transferred to cAMP-free medium, a germ tube emerged from each compartment. These results show that cAMP has two different effects on the development of hyphae: one is the promotion of branching, and the other is the suppression of polarized growth.

Key words Branching · cAMP · Hyphal growth · *Mucor globosus*

Hyphal branching is an important character for the development of a fungal colony. As a hypha elongates, new tips are formed near the apex of the tip by branching. Branching is affected by several environmental factors such as light, nutrients, and temperature (Lauter et al. 1998; Watters et al. 2000; Park et al. 2002). Regulators involved in polar tip growth, such as cytoskeletal components and secondary messengers, play important roles in branching. An actin mutant showed abnormal branching, indicating the importance of actin in branching (Virag and Griffiths 2003). Calcium ion promoted branch formation in *Neurospora* (Reissig and Kinney 1983). Cyclic adenosine 3', 5'-monophosphate (cAMP) also affects branching in several fungi, but the effect is opposite among different fungi. In *Neurospora*, cAMP-deficient mutants showed increased branching (Terenzi et al. 1974; Rosenberg and Pall 1979), indicating that cAMP suppresses branch formation. In *Phycomyces* and *Fusarium*, on the other hand, exogenous cAMP increased branching (Tu and Malhorta 1977; Robson et al. 1991).

Here, we examined the effect of cAMP on the branching of *Mucor globosus* A. Fisch. Many studies have been reported on the effect of cAMP on yeast-like growth in *Mucor* (Orlowski 1991), but its role in branching has not received as much attention. A few reports indicate the negative effect of cAMP on hyphal branching in *Mucor*. Lubbehusen et al. (2004) showed that cAMP-dependent protein kinase A suppressed hyphal branching. Pereyra et al. (2003) reported that the tripeptide Arg-Gly-Asp (RGD), which interacts with the integrin receptor in vertebrates, increased branching, and that cAMP analogue reduced branching by overriding the effect of RGD. In this article, we show that cAMP promoted branching but suppressed germination from spherical cells.

Mucor globosus (IFO 6745) was grown on MYC medium containing 1% malt extract, 0.2% yeast extract, 0.2% casamino acid, and 1.2% agar. Dilute spore suspension was dispersed over the surface of the agar medium. cAMP was dissolved in a few drops of distilled water and added to MYC medium to make the final concentration of 10mM. The plate was kept at 22°C. For the time-course experiment, the plate was placed on the stage of a microscope and photographed every 30min. Hyphal length and number of hyphal branch tips were determined on the photographs. Hyphal growth unit (HGU) is defined as follows (Caldwell and Trinci 1973): HGU = total length of a hypha/number of hyphal branch tips.

Cyclic AMP affected the growth pattern of the mycelia. Figure 1 shows representative growth patterns of the mycelia grown on a medium with or without cAMP. The mycelium grown on cAMP-free medium spread out more widely than that grown on cAMP-containing medium. Figure 2a,b shows the total hyphal length and the number of hyphal tips, respectively, in the mycelia grown on MYC agar medium with or without cAMP. Both the total hyphal length and the number of hyphal tips of the mycelium increased exponentially in both cAMP-treated and -untreated myce-

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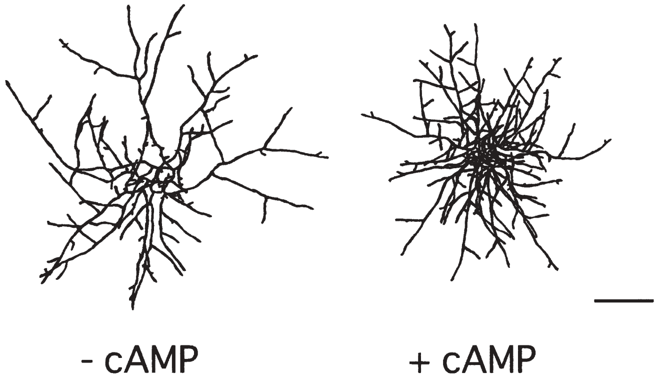

Fig. 1. Appearance of mycelia grown on cAMP-free (*–cAMP*) and cAMP-containing (*+cAMP*) medium for 15 h. *Bar* 500 μm

lia. However, the number of hyphal tips remarkably increased in the mycelium treated with cAMP. HGU of these mycelia are shown in Fig. 2c. When the number of branches per fixed hyphal length is increased, HGU is reduced (Caldwell and Trinci 1973). The length of HGU during the steady state was almost twice greater for the mycelium grown on normal medium than that for the mycelium grown on cAMP-containing medium, showing that cAMP promotes the branching.

On the other hand, in submerged culture, which is favored for arthrospore formation (Barrera 1983), cAMP promoted spherical growth. In cAMP-containing medium, some spores showed spherical growth (Fig. 3a) and others showed hyphal growth. cAMP promoted the septation of the hypha to form small compartments (Fig. 3b). Each compartment gradually swelled, just as does yeast. When these hyphae were transferred to cAMP-free medium, a germ tube emerged from each compartment, just like a normal spore (Fig. 3c). This observation suggests that cAMP suppresses the polarized growth in *M. globosus*, as reported in other *Mucor*.

These results show that cAMP has two different effects on the development of hyphae in *M. globosus*: one is the promotion of branching, and the other is the suppression of polarized growth. Branching and the initiation of polarized growth share a common process in forming new growing tips. cAMP seems to have different functions between branching and the initiation of polarized growth.

Our data showed that cAMP promoted branching in *M. globosus*. This result seems to be inconsistent with results that indicated a negative effect of cAMP on branching. Pereyra et al. (2003) reported that the RGD increased branching and the cAMP analogue reduced branching by overriding the effect of RGD. This result suggests that cAMP inhibits branching. However, all the germ tubes swelled with treatment with RGD, indicating that the germ tube may keep a spore-like nature. Therefore, the action of cAMP in the RGD-treated mycelia may be on the process that establishes polarized growth. The initiation of polarized growth was suppressed by cAMP in our experiment, also. That is, the different action of cAMP may be attributed to the difference in the process that is affected by cAMP, on the branching process in our experiment and on

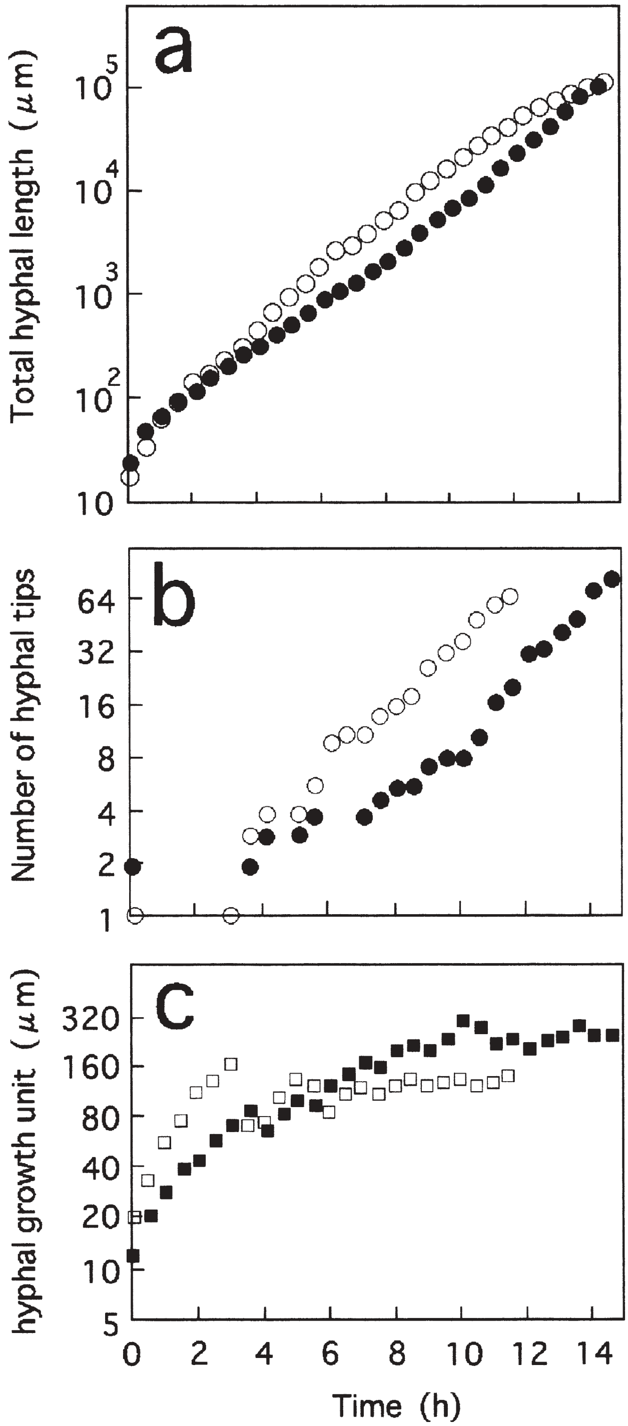

Fig. 2. Total hyphal length, number of hyphal tips, and length of hyphal growth unit for the mycelia grown on cAMP-free (*closed symbols*) and cAMP-containing (*open symbols*) medium

the process that establishes polarized growth in the RGD-treated mycelia.

Lubbehusen et al. (2004) also suggested a negative effect of cAMP on branching. They showed that overexpression of the regulatory subunit of PKA (PKAR) increased branching and discussed that the excess level of PKAR suppresses the cAMP-dependent protein kinase A cascade. In their

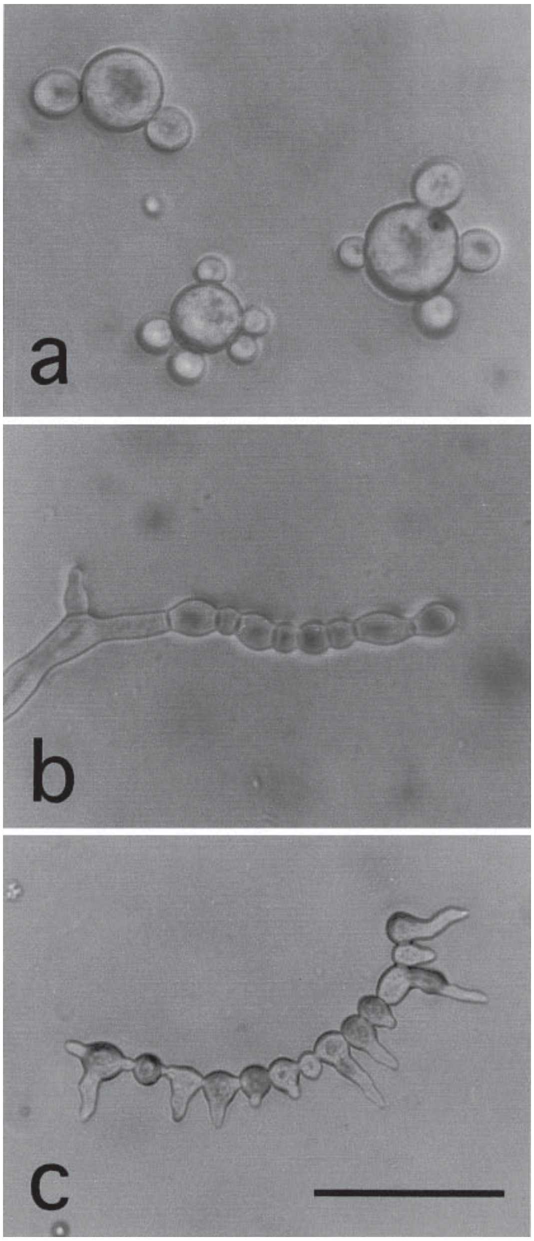

Fig. 3. Effect of cAMP on septation and swelling of hypha. **a** Yeast-like cells in cAMP-containing medium. **b** Hypha in cAMP-containing medium. **c** New hypha emerged from each compartment approximately 4 h after transferring to cAMP-free medium. *Bar* 100 μm

experiment, however, PKAC, which is the catalytic subunit of PKA, also increased. Because they did not examine the effect of cAMP on branching directly, cAMP might promote branching in their systems. Otherwise, the different effect might be ascribed to the species used in the experiments; that is, cAMP promotes branching in *M. globosus* but might suppress it in *M. circinelloides* and *M. rouxii*. Further study is necessary to clarify the function of cAMP on the growth of *Mucor*.

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