Morphology and Growth of a Temperature-sensitive Mutant of *Aspergillus nidulans* which Forms Aseptate Mycelia at Non-permissive Temperatures

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(Received 20 December 1978)

A study was made of the morphology and growth of young mycelia of a temperature-sensitive mutant (*sepA2*) of *Aspergillus nidulans*. At 25 °C the mutant grew at the same rate as the parental strain, formed septa and branched normally. At 30 and 37 °C the mutant grew at about three-quarters of the rate of the parental strain and, unlike the parental strain, branched dichotomously. The mutant was aseptate at 37 °C, and at 30 °C produced fewer septa than the parental strain. No septal initials were detected in mutant mycelia grown at 37 °C.

Aseptate mycelia of the mutant were grown at 37 °C and then transferred to 25 °C. After transfer to 25 °C, septa appeared parasynchronously throughout aseptate regions of the mycelium previously formed at 37 °C. The intercalary compartments formed in this manner had a mean length which was identical with those of intercalary compartments formed by the parental strain at 37 °C or the mutant at 25 °C. The lag between transfer of mutant mycelia from 37 to 25 °C and the first appearance of septa varied from 0.15 to 2.75 h; the maximum lag observed was similar to the organisms's doubling time (2.37 h) at 25 °C.

INTRODUCTION

A sequence of events (growth, mitosis and septation) occurs in the apical compartments (the terminal 'cells') of hyphae of *Aspergillus nidulans* and other fungi. These events, which are analogous to similar events in the cell cycle of unicellular organisms, form part of what in fungi has been called the duplication cycle (Trinci, 1978, 1979). In *A. nidulans* the duration of this cycle is identical with the organism's doubling time (Fiddy & Trinci, 1976). In *Basidiobolus ranarum* and *Schizophyllum commune* there are temporal, spatial and quantitative relationships between mitosis and septation. There is also a temporal relationship between mitosis and septation in *A. nidulans* but strict spatial and quantitative relationships between mitosis and septation are not observed; about 50 nuclei divide but a maximum of nine septa are formed in the rear half of the compartment. Apparently mitosis and septation are less tightly coupled in *A. nidulans* than in *B. ranarum* or *S. commune*.

Morris (1975) isolated five temperature-sensitive mutants of *A. nidulans* which failed to form septa at the restrictive temperature (42 °C). The mutants fell into at least three complementation groups. Two mutants had an otherwise normal morphology at the restrictive temperature but three formed hyphae which were wider than parental hyphae. The present paper describes the morphology and growth of young mycelia of the *sepA2* mutant.

METHODS

Organism and medium. The strains used in this study were the temperature-sensitive mutant UVts 402 carrying the gene *sepA2* (Morris, 1975) and the parental strain FGSC 154, which was obtained from the Fungal Genetics Stock Center of Humboldt State College, Arcata, California 95521, U.S.A. The growth medium contained (g l⁻¹): Oxoid yeast extract, 5; glucose, 10; Davis New Zealand agar, 15.

Plate cultures. Plates were overlaid with sterile cellophane (PT 325, British Cellophane) and inoculated with 0.1 ml of spore suspension in 0.1 % (v/v) Tween 80; the spores (about 100 to 200 per plate) were spread over the surface of the cellophane with a glass 'hockey stick'.

Coverslip cultures. A drop of spore suspension was placed on the centre of a 2.2 × 5 cm coverslip and covered with a square (about 1.5 × 1.5 cm) of cellophane. The cellophane was then overlaid with a thin (< 1 mm) layer of medium. The coverslip was inverted over a hole (about 2 × 3 cm) in a metal slide which also had a slit (about 1 mm wide) to allow aeration of the culture (Cole & Kendrick, 1968). The coverslip culture was completed by covering the base of the hole with another coverslip; both coverslips were fixed in place with vaseline. Coverslip cultures were placed on a glass rod and incubated in Petri dishes containing a few millilitres of water.

Mycelia growing in coverslip cultures were observed in a plane below the upper coverslip. They grew exponentially at the organism's maximum specific growth rate for the conditions (Fiddy & Trinci, 1976).

Observations on mycelia growing in coverslip and plate cultures. Some observations were made with a 35 mm Shackman Mark I time-lapse camera (Shackman & Sons, Chesham, Bucks.) and a Diapan Reichert microscope with × 25 and × 60 anoptral phase objectives.

The hyphal growth unit length (*G*) of a mycelium is the ratio between the length of the mycelium and its number of hyphal tips, i.e. it is the mean length of hyphae per tip. Time-lapse studies have shown that the length of the hyphal growth unit of a mycelium eventually attains a constant value (Trinci, 1974). This constant may also be determined from measurements made on populations of mycelia (Trinci, 1973). Hyphal growth unit measurements of the parental and mutant strains of *A. nidulans* were made on mycelia which had three to ten branches; populations of mycelia were grown as plate cultures.

Specific growth rates were calculated from measurements made on a time-lapse series of photographs of mycelia growing on plate cultures (Trinci, 1974).

The distance between successive septa (i.e. the length of successive intercalary compartments) in a germ tube of *A. nidulans* increases with distance from the spore, but eventually, when the germ tube attains a linear rate of growth, the hypha forms intercalary compartments of constant mean length (Fiddy & Trinci, 1976). Measurements of hyphal diameter and intercalary compartment length were made with an Olympus micrometer eyepiece on mycelia growing in coverslip cultures. The first four to ten intercalary compartments were measured in germ tube hyphae. No significant difference was observed between the mean lengths of intercalary compartments of different hyphae in the same treatment.

Electron microscopy. Mycelia were cultured on plates overlaid with cellophane. At intervals, the cellophane was removed and the mycelia were fixed in 2.5 % (v/v) glutaraldehyde in 0.1 M-phosphate buffer at 25 °C. The mycelia were post-fixed for 1 h at room temperature in 1 % (w/v) aqueous osmium tetroxide. After fixation, the mycelia were dehydrated, embedded, sectioned, stained and examined as described by Trinci & Collinge (1973).

RESULTS

Intercalary compartment length and hyphal diameter

At 30 °C and below, *sepA2* formed hyphae of approximately the same diameter as the parental strain. However, at 37 °C *sepA2* formed hyphae which were significantly ($P < 0.001$) wider than those of the parental strain at the same temperature (Table 1). At 25 °C and below, the mutant and parental strains formed intercalary compartments of approximately the same mean length. However, the mutant did not form septa at 37 °C, and at 30 °C formed intercalary compartments which were significantly ($P < 0.001$) longer than those of the parental strain at the same temperature (Table 1).

Hyphal growth unit length

Mycelia of the mutant and parental strains which formed septa eventually produced one branch per intercalary compartment. This relationship between septation and branch initiation has been observed previously in *A. nidulans* (Fiddy & Trinci, 1976).

Table 1. *Effect of temperature on hyphal diameter and intercalary compartment length of mycelia of the sepA2 mutant and the parental strain grown in coverslip cultures*

Temp. (°C)	Hyphal diameter* (μm)		Intercalary compartment length† (μm)	
	Parental strain	sepA2 mutant	Parental strain	sepA2 mutant
15	4.3 ± 0.7‡	4.3 ± 0.6	39 ± 17	36 ± 17
20	4.0 ± 0.6	4.1 ± 0.9	41 ± 23	36 ± 12
25	3.4 ± 0.7	3.7 ± 0.6	33 ± 14	34 ± 12
30	3.6 ± 0.6	3.8 ± 0.7	40 ± 16	58 ± 37
37	3.9 ± 0.7	5.3 ± 1.1	36 ± 16	Aseptate

* Each result is the mean of 15 to 24 hyphae.

† Each result is the mean of 21 to 54 compartments.

‡ Standard deviation of the sample.

Table 2. *Effect of temperature on hyphal growth unit length and specific growth rate of mycelia of the sepA2 mutant and the parental strain grown on plate cultures*

Temp. (°C)	Hyphal growth unit length* (G, μm)		Specific growth rate† (α, h ⁻¹)	
	Parental strain	sepA2 mutant	Parental strain	sepA2 mutant
15	130 ± 26‡	126 ± 24	Not determined	
20	161 ± 28	138 ± 29	Not determined	
25	140 ± 26	123 ± 27	0.302 ± 0.026	0.298 ± 0.020
30	155 ± 36	120 ± 29	0.540 ± 0.049	0.438 ± 0.090
37	115 ± 27	84 ± 22	0.572 ± 0.149	0.424 ± 0.080

* Each result is the mean of 16 to 47 mycelia.

† Each result is the mean of 6 to 10 determinations.

‡ Standard deviation of the sample.

Trinci (1973) showed that the length of the hyphal growth unit of mycelia of *Neurospora crassa* was not altered by temperature. Table 2 shows the effect of temperature on the length of the hyphal growth units of mycelia of the mutant and parental strains. Between 15 and 30 °C the hyphal growth unit length of both strains remained approximately constant, but both strains branched more profusely at 37 °C than at lower temperature, i.e. the length of their hyphal growth units were reduced at 37 °C. Thus, the absence of septa in *sepA2* at 37 °C had no *dramatic* effect on the frequency of branch initiation. Young mycelia of the parental strain formed lateral branches over the whole temperature range studied. Between 15 and 25 °C the mutant had an identical branching pattern to the parental strain, but at 30 and 37 °C it branched dichotomously as well as laterally (Fig. 1). Mycelia of *sepA2* grown at 37 °C for 5 d showed regions of sparse and dense dichotomous branching.

Specific growth rate

Table 2 also shows the effect of temperature on the specific growth rates of the parental and mutant strains. At 25 °C the mutant grew at the same rate as the parental strain, but at 30 and 37 °C it grew significantly ($P < 0.01$) more slowly than the parental strain.

Effect on specific growth rate and branching of transferring mycelia of *sepA2* from 25 to 37 °C

Figure 2 shows the growth of a germling (136 μm long at the time of transfer) which had been transferred from 25 to 37 °C. The mycelium grew exponentially (specific growth rate

Fig. 1. Branching patterns of mycelia of the *sepA2* mutant and the parental strain grown for 24 h at 37 °C.

Fig. 2. Growth and branching of a mycelium of the *sepA2* mutant which had been transferred from 25 to 37 °C: ○, mycelial length; ●, number of branches; ■, hyphal growth unit length.

of 0.29 h⁻¹) throughout the period of incubation at 37 °C. There was an initial decrease in the length of the hyphal growth unit after transfer to 37 °C. However, within about 5 h after transfer the hyphal growth unit had attained a constant value, i.e. mycelial length and number of branches were produced at the same specific rate. The branches formed soon after transfer to 37 °C were usually formed in a lateral position but subsequently dichoto-

Fig. 3. Septation in a mycelium of the *sepA2* mutant which had been grown at 37 °C and transferred to 25 °C: mycelium formed at 37 °C is shown by the solid line, mycelium formed at 25 °C is shown by the broken line. The location of septa formed at 25 °C is indicated. They were formed at the following times after transfer from 37 to 25 °C: A, 1.0 h; B, 1.17 h; C, 1.33 h; D, 1.62 h.

mous branching was more common. The initial reduction in hyphal growth unit length shown in Fig. 2 was not always observed after transfer of *sepA2* from 25 to 37 °C.

Effect on hyphal extension, branching and septation of transferring mycelia of sepA2 from 37 to 25 °C

Aseptate germlings of *sepA2* were grown in coverslip cultures at 37 °C until they were about 0.5 to 1 mm long. They were then transferred to 25 °C. After a lag, which varied from 0.15 to 2.75 h (mean of 1.39 ± 0.97 for ten germlings), septa were produced in regions of the germlings which had been formed previously at 37 °C. Septa formed after transfer from 37 to 25 °C were produced parasynchronously; up to six septa were produced in each germling within 20 min of transfer to 25 °C. However, when somewhat larger aseptate mycelia were grown at 37 °C and then transferred to 25 °C, the septa formed at the lower temperature were produced even less synchronously (Fig. 3) than in germlings. For example, the 37 septa formed in the mycelium shown in Fig. 3 appeared over a period of 37 min; this mycelium had a total length of about 2.5 mm at the time of transfer. However, Fig. 3 shows that septation was synchronized locally within such larger mycelia.

Septation occurs at a specific point in the duplication cycle of apical compartments of leading hyphae of *A. nidulans* and the interval between successive septation cycles is the same as the organism's doubling time (Trinci, 1979). The mean doubling time of *sepA2* at 25 °C was 2.37 ± 0.15 h. This value may be compared with the interval observed (2.3 h) between the formation of two successive groups of septa in a germling of *sepA2* (about 0.5 mm long at the time of transfer) transferred from 37 to 25 °C. Further, the maximum lag (2.75 h) observed between transfer of *sepA2* from 37 to 25 °C and the appearance of septa is also similar to the organism's doubling time at 25 °C.

The intercalary compartments formed in mycelia transferred from 37 to 25 °C had a mean

Fig. 4. Dichotomous branching of a hypha of the *sepA2* mutant which had been transferred from 37 to 25 °C.

length of $35 \pm 14 \mu\text{m}$ (54 compartments were measured). This value should be compared with the lengths of intercalary compartments formed by the parental strain at 37 °C ($36 \pm 16 \mu\text{m}$) and the mutant strain at 25 °C ($34 \pm 12 \mu\text{m}$).

Figure 4 shows growth of a hypha of *sepA2* which had been transferred from 37 to 25 °C. There was usually a deceleration in the rate of hyphal extension after transfer to 25 °C and sometimes the tip branched dichotomously (Figs 3 and 4). Hyphae formed at 25 °C were always narrower than the parental hyphae formed at 37 °C from which they arose (Fig. 4); this reduction in hyphal diameter occurred almost immediately after transfer from 37 to 25 °C.

Ultrastructure of parental and sepA2 mycelia grown at 37 °C

Most of the hyphae of mycelia of *sepA2* grown at 37 °C for 21.5 h appeared to have an ultrastructure similar to that of the parental strain grown for the same time at the same temperature. However, judged by the criteria established by Trinci & Righelato (1970), hyphae in some sections of the mutant strain showed signs of autolysis. Vesicles were observed at the tips of hyphae of both the parental (mean vesicle diameter, $80 \pm 13 \text{ nm}$) and mutant (mean vesicle diameter, $76 \pm 9 \text{ nm}$) strains. The walls of hyphae of the mutant strain ($141 \pm 47 \text{ nm}$) were thicker than those of the parental strain ($91 \pm 46 \text{ nm}$), possibly as a result of unbalanced wall growth. Septa were frequently observed in the parental strain but never in the mutant strain; no septal primordia were observed in the mutant strain.

DISCUSSION

Temperature-sensitive mutants of *Escherichia coli* have been isolated which form long, aseptate filaments at the restrictive temperature (42 °C) (Reeve *et al.*, 1970; Reeve & Clark, 1972; Allen *et al.*, 1974). When bacterial filaments formed in this way are transferred to permissive temperatures (28 or 30 °C) they rapidly form septa and the organism eventually returns to its normal size. Reeve & Clark (1972) suggest that septation sites are produced by these mutants at permissive and restrictive temperatures but that these sites are expressed only at permissive temperatures. The same hypothesis may be advanced to explain the behaviour of the *sepA2* mutant of *A. nidulans*. This is suggested by the observation that aseptate mycelia grown at 37 °C and transferred to 25 °C very quickly generate a normal distribution of septa (Fig. 3). Although we were unable to detect morphologically identifiable septal initials in mycelia of *sepA2* grown at 37 °C, septal primordia must presumably exist at the biochemical level.

It has been shown that septation in apical compartments of germ tube or leading hyphae of *A. nidulans* occurs at a specific point in the duplication cycle and follows mitosis (Trinci, 1978, 1979). There is evidence (Morris, 1975) that mitosis in aseptate mycelia of the *sepA2* mutant is synchronized and synchronous mitosis has been observed in short germlings of *A. nidulans* (Rosenberger & Kessel, 1967). Thus, after transfer of *sepA2* mycelia from 37 to

25 °C, cross-wall formation may occur when the 'septation' point in the duplication cycle is first attained. This hypothesis is supported by the observation that the lag between transfer from 37 to 25 °C and the onset of septation varied from a few minutes to approximately the organism's doubling time at 25 °C. This variation in the duration of the lag may reflect a variation in the stages of the duplication cycle attained by the germlings at the time of transfer. In addition, the mean lag (1.39 h) observed between transfer of *sepA2* from 37 to 25 °C and the onset of septation is approximately half the organism's doubling time at 25 °C (2.37 h); this result would be predicted by the hypothesis. Thus it appears that when the 'septation' point in the duplication cycle is first attained on transfer to 25 °C, septal primordia formed in previous duplication cycles at 37 °C are usually recognized and expressed. The synchrony observed in the formation of septa in germlings of *sepA2* transferred from 37 to 25 °C varied with germling length; septation was less synchronized in large (Fig. 3) than in small germlings. Rosenberger & Kessel (1967) and Fiddy & Trinci (1976) showed that mitotic synchrony decayed rapidly when septated germlings of *A. nidulans* increased in size. However, synchronous mitosis in aseptate mycelia of *sepA2* may be maintained for a somewhat longer period than in septated mycelia of this organism (Morris, 1975).

The existence of the *sepA2* mutant indicates that septal wall growth may be divorced from hyphal wall growth and shows that an independent mechanism regulates septal formation. The existence of the mutant also indicates that the correct placing of septa does not depend on their position relative to existing septa and that the frequency of branch initiation, as distinct from their location, is not regulated by septa. Our observations suggest that the presence of septa influences the location of branches, since the parental strain formed lateral branches whilst the mutant strain at 30 or 37 °C tended to branch dichotomously. Trinci (1974) and Prosser & Trinci (1979) have suggested how septation may influence branch location.

We thank Annette J. Collinge for her capable assistance with the electron microscopy.

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