Mitosis, Septation, Branching and the Duplication Cycle in *Aspergillus nidulans*

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SUMMARY

Mitosis, septation and branching were studied in undifferentiated mycelia and leading hyphae of *Aspergillus nidulans*, a mould which forms incomplete septa. After spore germination, nuclei divided synchronously until germ-tube hyphae contained 8 or 16 nuclei; mitosis occurred when the volume of cytoplasm per haploid nucleus was about $60 \mu\text{m}^3$. Intercompartment development was not synchronized, consequently mitosis in the mycelium as a whole eventually became asynchronous. During the stage of asynchronous compartment development, the nuclei, septa, branches and total length of undifferentiated mycelia all increased exponentially at approximately the same specific rate.

Septa were formed in hyphae in groups of up to nine; the mean time required for the formation of a group of septa was about 9 min. The mean interval between successive cycles of septation in a hypha was approximately the same as the organism's doubling time. There was a highly significant correlation coefficient between septation and branch initiation and most intercalary compartments initially formed a single branch.

The volume of cytoplasm per nucleus in a diploid strain was approximately double the value observed for a haploid strain. However, the length of the hyphal growth unit was not affected by ploidy.

The study suggests that a duplication cycle can be recognized during mycelial growth which is analogous to the cell cycle observed in unicellular micro-organisms.

INTRODUCTION

Under optimal conditions, growth of an asynchronous population is 'balanced', i.e. all extensive properties of the population increase at the same rate (Campbell, 1957). The intermittent synthesis of certain macromolecules, enzymes and organelles observed during growth of an individual cell (Mitchison, 1971) is obscured in such populations. Evidence for intermittent synthesis in the cell cycle is usually obtained from studies of single cells or synchronized populations.

Regulatory mechanisms must be present in moulds to ensure that organelles and macromolecules are synthesized in an integrated fashion. The existence of such mechanisms is suggested by the more or less homogeneous distribution of most organelles, macromolecules and biosynthetic functions observed during 'balanced' growth of mycelia. Fencel, Machek & Novak (1969), for example, found that during exponential growth, RNA synthesis was evenly distributed throughout mycelia of *Aspergillus niger*. However, organelles (e.g. apical vesicles; Grove & Bracker, 1970) and enzyme activity (e.g. chitin synthetase; Gooday, 1971) associated with tip extension have a heterogeneous distribution in mycelia.

Events may occur during mycelial growth which are analogous to those observed during

the cell cycle of unicellular organisms. Since cell separation does not occur in moulds, the term 'duplication cycle' will be used to describe these events. The duration of a duplication cycle will be given by the mould's doubling time and during a cycle there will be a doubling of all the constituent components of the protoplasm. The existence of such a cycle is suggested by the work of King & Alexander (1969) and Clutterbuck (1970).

Steele & Trinci (1975) compared the morphology and growth kinetics of hyphae of undifferentiated and differentiated (colonies) mycelia. Undifferentiated mycelia have a total hyphal length of only a few millimetres and grow exponentially under the near constant conditions which prevail during the early stages of growth on solid media or submerged culture. The rates of extension of most of the branches of such mycelia accelerate towards the linear growth rate characteristic of the strain and conditions (see Fig. 7 and Table 3, Trinci, 1974). Leading hyphae of differentiated mycelia extend at a linear rate under the near constant conditions which prevail at the colony's margin.

The present study was undertaken to determine the relationships between growth, nuclear division, septation and branch initiation in undifferentiated mycelia and leading hyphae of *Aspergillus nidulans*. It was hoped that the investigation would establish the presence of a duplication cycle in *A. nidulans*.

METHODS

Organisms and media. The strains used were *Aspergillus nidulans* BWB224 vey and a diploid formed from *A. nidulans* BWB199 and BWB229 given to us by Dr B.W. Bainbridge. The haploid *A. nidulans* BWB224 was grown on Oxoid malt extract agar, and the diploid on DM medium (Trinci, 1971) from which the vitamins were omitted. The haploid strain was grown on malt extract agar because on this medium the hyphae had a wider diameter which enabled the septa to be seen more clearly.

Cultural conditions. All cultures were grown and observed at 25°C. Hyphae to be stained with Giemsa were grown on slide cultures using the method of Dr B. W. Bainbridge. A sterile microscope slide was coated with 10% (w/v) crystallized egg albumen (preserved with a crystal of phenol) and allowed to dry. The slide was then inoculated with a spore suspension of *A. nidulans* and covered with a piece of sterile cellophane (PT 300, British Cellophane). Medium (1 ml) at 45 to 50°C was pipetted on to the surface of the cellophane. Slides were incubated in a Petri dish resting on a bent glass rod over water. Before staining, the cellophane and medium were carefully removed.

For observations of septation, *A. nidulans* was grown in 'coverslip' cultures. A sterile 22 × 50 mm coverslip was coated with 10% (w/v) egg albumen and allowed to dry. A drop of spore suspension placed on the centre of the coverslip was covered with a small piece of cellophane and sufficient medium was pipetted on top to just cover the cellophane. The culture was incubated in a Petri dish standing on a glass rod over water. When the culture was ready for observation, the coverslip was inverted over a metal slide which had a central circular hole (diam. 2 cm) and a slit to allow aeration of the culture, as described by Cole & Kendrick (1968). Another coverslip covered the base of the hole. Both coverslips were sealed with paraffin wax. Growth of the hyphae was observed in a single plane below the upper coverslip.

For studies of septation in leading hyphae, this method was modified by covering the whole coverslip with medium and inoculating across it in a line. The culture was then incubated as described above. Just before making observations, the medium and some of the hyphae were cut away with a razor blade so that only a small area of medium containing leading hyphae was left. Cultures were prepared for observation as described above, and

Fig. 1. Growth of mycelia of *A. nidulans* in slide (●) and coverslip (○) cultures at 25°C on malt extract agar.

observations were initiated about 1 h after the hyphae had been transferred to the slide cultures.

Observations on the sites of branch initiation were made on undifferentiated mycelia grown in 9 cm Petri dishes containing 15 ml medium overlaid with cellophane.

Nuclear staining. The slides supporting the mycelia were cold treated at -20°C in a deep freeze for 10 to 30 min to cause the nuclei to round up (as suggested by Clutterbuck & Roper, 1966). They were stained by a method suggested by Dr B. W. Bainbridge. The hyphae were fixed in freshly-prepared acetic acid/alcohol (3:1, v/v) for 30 s, then hydrolysed for 12 to 14 min in 1 M-HCl at 60°C , rinsed in tap water and stained for 40 to 50 min with Gurr's Giemsa diluted 1 in 10 with 0.05 M-phosphate buffer at pH 6.8. The quality of nuclear staining was checked and overstained preparations were differentiated in dilute acetic acid (1 loopful in 90 ml water). The slides were air dried and mounted in DPX (Edward Gurr, London SW14).

Measurements were made using a micrometer eyepiece or from camera lucida drawings.

Ratio of hyphal length to nuclear number (H_n). Since there is little variation in the diameter of hyphae of undifferentiated mycelia (Trinci, 1973; Steele & Trinci, 1975) the ratio of total hyphal length of the mycelium to the number of nuclei which it contains provides a convenient measure of the relative volume of cytoplasm per nucleus in the mycelium. This ratio is designated the H_n value of the mycelium and is expressed in μm . If the mean hyphal diameter is known, H_n can be used to estimate the actual volume of cytoplasm per nucleus. However, such calculations arbitrarily ignore cytoplasmic vacuolation.

Septation and branching. Some observations were made with a 35 mm Shackman Mark I time-lapse camera (Shackman & Sons, Chesham, Buckinghamshire). Other studies were made by direct observations using a micrometer eyepiece.

All observations were made on sparse hyphal systems to avoid problems which might arise from growth becoming oxygen limited.

RESULTS

Development of undifferentiated mycelia

Growth kinetics. The lengths of mycelia of *A. nidulans* grown in slide and coverslip cultures increased exponentially (Fig. 1) until the total hyphal lengths were at least 3 mm; mycelia had specific growth rates of $0.32 \pm 0.06 \text{ h}^{-1}$ in slide cultures and $0.34 \pm 0.03 \text{ h}^{-1}$ in coverslip

Fig. 2

Fig. 3

Fig. 2. Total mycelial length, number of nuclei (●), number of hyphal tips (○) and number of septa (■) of haploid mycelia of *A. nidulans* grown in slide cultures at 25°C on malt extract agar. The numbers of septa are the means for groups of mycelia which varied in length by 20 μm ; each point is the mean of 1 to 14 mycelia. The numbers of hyphal tips are the means for groups of mycelia which varied in length by 40 μm ; each point is the mean of 1 to 35 mycelia.

Fig. 3. Total mycelial length, number of nuclei (●), number of hyphal tips (○) and number of septa (■) of diploid mycelia of *A. nidulans* grown in slide cultures at 25°C on DM medium. The numbers of septa are the means for groups of mycelia which varied in length by 20 μm ; each point is the mean of 1 to 11 mycelia. The numbers of hyphal tips are the means for groups of mycelia which varied in length by 40 μm ; each point is the mean of 1 to 14 mycelia.

cultures. The exponential kinetics observed suggest that growth under both cultural conditions was not oxygen limited.

The conidia of *A. nidulans* are uninucleate. In Fig. 2 the numbers of nuclei, hyphal tips and septa of mycelia grown in slide cultures are plotted against the total mycelial length. Since mycelia grew exponentially, the total mycelial length provides a measure of the relative stage of mycelial development, i.e. the period since spore germination. Thus, Fig. 2 provides an insight into the development of a single mycelium as well as providing information about the population actually studied.

Nuclei. After spore germination, nuclei initially divided synchronously but subsequently mitosis in the mycelium as a whole became asynchronous (Fig. 2). The degree of synchrony during the early stages of mycelial growth is underestimated in Fig. 2 because some points overlap others on the plateaux. The transition from synchronous to asynchronous mitosis was apparently initiated in the haploid strain when the mycelia were about 60 to 130 μm long and contained 8 or 16 nuclei, i.e. after three or four cycles of nuclear division. The nuclei in the diploid strain divided synchronously until mycelia contained 16 nuclei and were about 500 μm long (Fig. 3).

The minimum and maximum lengths observed for mycelia containing 4, 8, 16 and 32 nuclei are given in Table 1 together with their H_n values. The maximum lengths attained by mycelia containing these numbers of nuclei were approximately twice the minimum mycelial lengths observed for the same numbers of nuclei (Table 1). Mitosis occurred when the cytoplasmic volume per nucleus attained a mean value of about 57 μm^3 (Fig. 2; Table 1). The

Table 1. *Minimum and maximum hyphal lengths observed for mycelia containing 4 to 32 nuclei and the corresponding ratios of hyphal length to nuclear number (H_n)*

Haploid mycelia were grown on malt extract agar in slide cultures at 25°C.

		No. of nuclei in mycelium			
		4	8	16	32
Total mycelial length (μm)	{ Minimum value	9	29	58	120
	{ Maximum value	26	56	126	230
Mean hyphal length per nucleus, H_n (μm)	{ Minimum value	2.3	3.6	3.6	3.8
	{ Maximum value	6.5	7.0	7.9	7.2

Mean = 3.3*

Mean = 7.2*

* H_n values of 3.3 and 7.2 give values of cytoplasmic volume per nucleus of 26 and 57 μm^3 respectively.

Table 2. *Kinetics of the development of undifferentiated mycelia of the haploid strain of *A. nidulans* grown on malt extract agar in slide cultures at 25°C*

Parameter measured	Doubling time, T_d (h)	Specific growth rate, α (h^{-1})
Total hyphal length	2.2	0.32 ± 0.06
No. of tips†	2.7*	0.26*
No. of nuclei‡	2.5*	0.28*
No. of septa§	2.2*	0.32*

* Estimated from the data shown in Fig. 2.

Calculated for mycelia with a total hyphal length of † 100 to 860 μm , ‡ 8 to 740 μm , and § 70 to 880 μm .

Table 3. *Numbers of nuclei and H_n values of apical and intercalary compartments of undifferentiated mycelia* of *A. nidulans* grown on malt extract agar in slide cultures at 25°C*

	Apical compartment	Intercalary compartment
Mean length (μm)	127 ± 44	26 ± 10
Mean no. of nuclei	20 ± 7	4 ± 2
Range of no. of nuclei observed	10 to 43	2 to 10
Mean hyphal length per nucleus, H_n (μm)	6.9 ± 1.7	6.6 ± 1.2

* The mycelia in the sample varied in length from 10 to 750 μm and contained 2 to about 100 nuclei.

numbers of nuclei in the haploid strain increased exponentially at a rate estimated to be similar to the rate of increase in mycelial length (Table 2).

The numbers of nuclei and H_n values of apical and intercalary compartments of undifferentiated, haploid mycelia are given in Table 3; a sample of 171 mycelia, which varied in length from 10 to 750 μm , had an H_n value of $5.7 \pm 1.2 \mu\text{m}$ whereas a sample of 92 mycelia of the diploid strain, which varied in length from 12 to 680 μm , had an H_n value of $22 \pm 7 \mu\text{m}$. Hyphae of unfixed mycelia of the haploid and diploid strains had mean diameters of 3.2 and 2.6 μm respectively. It was calculated that the haploid and diploid strains had mean values of the volume of cytoplasm per nucleus of 45 and 117 μm^3 respectively.

The nuclei of both strains were distributed more or less uniformly throughout mycelia (Fig. 4), although nuclei were sometimes observed in pairs as if they had recently undergone mitosis. The mean distance from the hyphal tip to the first nucleus in a sample of 42 apical compartments (mean length $127 \pm 44 \mu\text{m}$) of the haploid strain was $6.9 \pm 1.7 \mu\text{m}$, i.e. the same as the mean distance between adjacent nuclei in apical compartments (Table 3). The distance between the tip and the first nucleus varied from 3.2 to 18.2 μm and increased with

Fig. 4. Camera lucida drawing of haploid (a to e) and diploid (f) mycelia grown in slide cultures at 25°C and stained with Giemsa.

Fig. 5. Septation and branching in a germ-tube hypha grown in a coverslip culture at 25°C on malt extract agar. Tracings made from photographs. Septa belonging to one group are indicated by the letters A, B and C, and the sequence of septation within a group is indicated by the numbers.

compartment length; there was a highly significant ($P = <0.001$) correlation coefficient (0.77) between apical compartment length and distance from the tip to the first nucleus.

Septa. After an initial period, the number of septa in undifferentiated mycelia of *A. nidulans* increased exponentially (Fig. 2) at a rate similar to the rate of increase in mycelial length (Table 2). The first septum was usually formed at the base of the germ tube (Fig. 4) and was first observed in hyphae containing 4 or 8 nuclei. The second septum was usually

Table 4. *Septation in germ-tube hyphae of undifferentiated mycelia and leading hyphae of A. nidulans grown on malt extract agar at 25°C in coverslip cultures*

	Germ-tube hyphae	Leading hyphae
Mycelial length doubling time, T_d (h)	2.1 ± 0.2	—
Interval between the formation of the first septum in one group and the first septum in the next group in the same hypha (h)	1.8 ± 0.3	2.1 ± 0.2
Mean no. of septa formed in a group during one cycle of septation	3.4 ± 1.7	4.1 ± 1.1
Mean time to form a group of septa (min)	8.6 ± 6.3	8.5 ± 6.0
Lengths (μm) of the proximal (septated region) and the distal (new apical compartment) regions into which apical compartments were divided just after a period of septation	<div> <div></div> <div> Distal 185 ± 52 Proximal 123 ± 51 </div> </div>	<div> <div></div> <div> 240 ± 34 231 ± 41 </div> </div>

formed when germ-tube hyphae were about 70 to 150 μm long and contained 16 nuclei (Figs 2 and 4). Thus after four cycles of nuclear division usually only two septa had been formed.

There was a linear relationship between the length of mycelia and the number of septa which they contained; the correlation coefficient (0.97) between these two parameters was highly significant ($P = < 0.001$).

Studies were made of septation in apical compartments of germ-tube hyphae of undifferentiated mycelia of *A. nidulans* grown in coverslip cultures. A group of 1 to 9 septa was formed in an apical compartment during a single period of septation (Fig. 5; Table 4). There was a mean interval of 8.6 ± 6.3 min (range 2 to 30 min) between the formation of the first and last septum in a group. Septa in a single group were sometimes formed sequentially, with the septum nearest the tip being formed last, but frequently two or more septa were formed simultaneously or one or more septa was formed out of order (Fig. 5). There was a mean interval of 1.8 ± 0.3 h between successive cycles of septation in germ-tube hyphae (Fig. 5; Table 4). A newly-formed group of septa usually divided the apical compartment into proximal (septated) and distal regions of unequal length (Table 4), with the usually longer distal region forming the new apical compartment of the hypha. Figure 6a shows several cycles of septation in a germ-tube hypha which had an initial length of about 200 μm . The length of the apical compartment just after a period of septation (i.e. its minimum length) and the length of the same compartment just before the next period of septation (i.e. its maximum length) increased with hyphal length and hence time (Fig. 6a). This increase in the minimum and maximum lengths of successive apical compartments is shown in Fig. 7. Apical compartments attained their maximum values for these parameters when the germ-tube hyphae were about 800 μm long.

Septation cycles in apical compartments of germ tubes formed by the same spore were observed to be out of phase by up to 53 min. Such observations suggest that septation cycles (and hence duplication cycles) in apical compartments of a single mycelium were not always synchronized and accounts for the apparent decay in synchrony of mitosis which followed division of the coenocyte into compartments by septation (Fig. 2).

Branch initiation and growth. Trinci (1974) showed that the number of branches in undifferentiated mycelia of *Aspergillus nidulans* increases at an exponential rate. In the present study it was found that, after an initial period, the number of branches increased exponentially at more or less the same specific growth rate as the other parameters measured (Fig. 2;

Fig. 6

Fig. 7

Fig. 6. Variation in the length of the apical compartments of (a) a germ-tube hypha and (b) a leading hypha at the margin of a colony. Coverslip cultures were grown at 25°C on malt extract agar. The extension rate of the germ-tube hypha increased throughout the observation period; that of the leading hypha was constant. Periods of septation and the numbers of septa formed are indicated by arrows.

Fig. 7. Variation in the minimum (○) and maximum (●) lengths of successive apical compartments formed by germ-tube hyphae of *A. nidulans* grown in coverslip cultures at 25°C on malt extract agar. Each point is the mean of the lengths of three to nine apical compartments.

Fig. 8. Relationship between septation and branch initiation in an undifferentiated mycelium of *A. nidulans* grown at 25°C in a Petri dish containing malt extract agar overlaid with cellophane. Tracing made from photographs. The bars indicate the positions of septa.

Table 2). The populations of haploid and diploid mycelia shown in Figs 2 and 3 had hyphal growth units (Trinci, 1974) of 73 ± 23 and 121 ± 53 μm respectively. However, the strains were grown on different media and medium composition can affect hyphal growth unit length (Bull & Trinci, 1976). When the haploid strain was grown on DM medium (i.e. the same medium as the diploid) it had a hyphal growth unit of 118 ± 24 μm . Thus the length of the hyphal growth unit of a mould is not affected by its ploidy.

There was a highly significant ($P = < 0.001$) correlation coefficient (0.92) between the number of septa and the number of branches produced by mycelia; these parameters varied

Table 5. *Branching pattern of undifferentiated mycelia of A. nidulans grown for 24 h at 25°C on malt extract agar plates covered with cellophane*

Each germ-tube hypha had formed nine intercalary compartments between the spore and the hyphal tip.

Compartment*	No. of hyphae with :			
	No branches	One branch	Two branches	Three branches
1	0	7	0	0
2	0	7	0	0
3	0	7	0	0
4	0	7	0	0
5	1	4	1	1
6	0	6	1	0
7	2	4	1	0
8	3	4	0	0
9	5	2	0	0
Apical compartment	1	4	2	0

* Numbered from spore to hyphal tip.

Table 6. *Site of branch initiation from intercalary compartments of hyphae of undifferentiated mycelia and leading hyphae of A. nidulans grown on malt extract agar at 25°C*

The number of branches in each compartment region is expressed as a percentage of the total number of branches.

Region of intercalary compartment* . . .	No. of branches (%) in each region				
	1	2	3	4	5
Intercalary compartments of hyphae of undifferentiated mycelia	25	29	17	18	12
Intercalary compartments of leading hyphae of differentiated mycelia (colonies)	27	41	17	10	5

* Each compartment was divided, visually, along its length into five equal regions, 1 being nearest the hyphal tip.

linearly. Each intercalary compartment usually produced a single branch initially (Fig. 8). Table 5 shows the branching patterns of seven germ-tube hyphae which had each formed nine intercalary compartments. In all germ tubes the four compartments adjacent to the spore had each formed a single branch. However, some compartments closer to the tip had either not formed branches or had produced two branches per compartment. Branches were also sometimes formed by apical compartments. It is possible that one of the two branches of some intercalary compartments may initially have been formed when that segment of the hypha formed part of the apical compartment, i.e. they were originally branches of the apical compartments. The position of branch initiation from intercalary compartments is shown in Table 6; branches were initiated more frequently from proximal (with respect to the hyphal tip) than from distal regions of compartments. Changing the incubation temperature over the range 25 to 37°C did not influence the site of branch initiation.

There was a lag of 50 ± 40 min (range 5 to 110 min) between the completion of a septum and the initiation of a branch from the intercalary compartment distal to the septum (Fig. 9). The branches produced after formation of a group of septa in a hypha did not appear to be initiated in any particular sequence; the lag observed between septation and initiation of a branch behind the septum was approximately the same for those septa in a group which were nearest and furthest away from the hyphal tip.

There was a lag of 6.4 ± 1.5 h between the initiation of the first and second branches

Fig. 9. Formation of first and second branches produced by an intercalary compartment of *A. nidulans* grown at 25°C on malt extract agar in a coverslip culture. Tracings made from photographs.

Fig. 10. Variation in the rate of extension of (a) a germ tube and (b) a branch of an undifferentiated mycelium of *A. nidulans*. Coverslip cultures were grown at 25°C on malt extract agar.

formed by intercalary compartments; the second branch was usually only initiated after a septum had formed at the base of the first branch (Fig. 9). There was a lag of 2.5 ± 1.5 h between the formation of a septum at the base of the first branch and the initiation of a second branch from the parent compartment. The formation of septa at the base of branches (Fig. 9) was reminiscent of the septa formed at the base of germ-tube hyphae (Fig. 4).

Germ-tube hyphae and branches of undifferentiated mycelia attained their maximum rates of extension (about $100 \mu\text{m h}^{-1}$) when they were about 600 and 400 μm long respectively (Fig. 10); these lengths represent the *maximum* lengths of the peripheral growth zones of these hyphae (Trinci, 1974). Fiddy & Trinci (1976) have shown that in *Geotrichum candidum* a primary branch and its parent intercalary compartment initially increase in length at an exponential rate. Thus

$$\ln L_1 = \ln L_0 + \alpha(t_1 - t_0) \quad (1)$$

in which L_0 is the combined length of the branch and its parent intercalary compartment at

Fig. 11. (a) Growth (○) of a primary branch and its parent intercalary compartment (34 µm long). The solid line shows branch growth as predicted by equation (1).

(b) Growth (●) of the same primary branch, assuming that a 50 µm length of parent hypha contributed to its extension. The solid line shows the growth of this branch as predicted by equation (1). Extension rate of the same primary branch (▲).

time t_0 , L_1 is the combined length of the branch and compartment at time t_1 , and α is the mould's specific growth rate.

Figure 11a compares the observed growth of a primary branch of *A. nidulans* and its parent intercalary compartment (which was 34 µm long) with their growth as predicted by equation (1). Most of the branches studied behaved in a similar manner to that illustrated in Fig. 11. The observation that primary branches grow at a faster rate than predicted by equation (1) suggests that their growth may initially be supported by a region of hypha in excess of the parent intercalary compartment. Since septa of *A. nidulans* are incomplete, it is certainly possible that regions of the parent hypha in addition to the intercalary compartment may contribute to branch growth. When it was assumed that a 50 µm (instead of 34 µm)

Table 7. Length, number of nuclei, H_n value and distance between nuclei in apical compartments of leading hyphae of *A. nidulans* grown in slide cultures at 25°C on defined medium containing glucose (0.2 g l⁻¹)

Each result is the mean of results for 14 apical compartments.

Apical compartment length (μm)	391 $\pm$ 106
No. of nuclei per compartment	52 $\pm$ 15
Hyphal length per nucleus, H_n (μm)	8.7 $\pm$ 2.9
Distance* from hyphal tip to first nucleus (μm)	17.5 $\pm$ 5.2
Mean distance* between the apical 10 nuclei in a compartment (μm)	5.4 $\pm$ 3.0
Mean distance* between the middle 10 nuclei in a compartment (μm)	12.0 $\pm$ 5.7
Mean distance* between the distal 10 nuclei in a compartment (μm)	15.6 $\pm$ 6.2
Distance* from the distal nucleus to the septum delimiting the compartment (μm)	37.6 $\pm$ 6.3

* Some nuclei were very elongated; measurements were made from the middle of one nucleus to the middle of the next or from the middle of the proximal or distal nucleus to septum or the hyphal tip.

portion of the parent hypha supported growth of the branch shown in Fig. 11*a*, the kinetics of branch growth were adequately described (Fig. 11*b*) by equation (1). Growth of three (out of four) other branches also followed the kinetics described by equation (1) when it was assumed that initially their growth was supported by a 10 to 14 μm length of parent hypha in addition to their intercalary compartments. Six to 8 h after the initiation of a branch, branch growth deviated from the kinetics predicted by equation (1) (Fig. 11*b*). Such deviations were first observed when the branches and their parent hyphal portions had attained lengths of about 330 to 370 μm ; these lengths may represent the length of the peripheral growth zones of the branches. It is probably significant that there was an interval of about 6.4 h (see above) between the initiation of the first and second branches formed by an intercalary compartment, i.e. growth of the branch started to deviate from the predicted line (Fig. 11*b*) at about the time when the parent intercalary compartment produced a second branch (Fig. 9) and hence probably ceased to contribute to growth of the first branch. Also primary branches started to decelerate from their previous exponential rate of growth at about the same time as they attained a linear rate of extension (Fig. 11*b*). Primary branches produced secondary branches (i.e. the primary branches themselves formed branches) when they were about 460 to 619 μm long (Fig. 11*b*); secondary branches were produced at sites which were 41 to 55 μm from the base of the primary branches. These observations suggest that as primary branches increased in length, portions of hypha were progressively removed from the rear of their peripheral growth zones (Trinci, 1971).

Leading hyphae of differentiated mycelia (colonies)

The apical and intercalary compartments (283 $\pm$ 56 and 55 $\pm$ 17 μm respectively) of leading hyphae grown on malt extract agar were longer than the equivalent compartments of undifferentiated mycelia (Table 3). Septa were formed in apical compartments of leading hyphae in groups of between two and six. Clutterbuck (1970) has also observed multiple septation in leading hyphae of *A. nidulans*. There was an interval of 8.5 $\pm$ 6.0 min (range 2 to 20 min) between the formation of the first and last septum of a group and an interval of 2.1 $\pm$ 0.2 h between successive cycles of septation in a hypha (Table 4; Fig. 6*b*). A newly-formed group of septa divided the apical compartment into a proximal septated region and a non-septated distal region (the new apical compartment) of almost equal length (Fig. 6*b*; Table 4).

Leading hyphae were photographed every minute for about 5 h (i.e. during at least two septation cycles); differences in extension rate of about $0.6 \mu\text{m min}^{-1}$ could be detected at the magnification employed. The apical compartments maintained a constant rate of extension throughout the observation period showing that there was no deceleration in extension rate associated with septation.

The number and distribution of nuclei in apical compartments of leading hyphae of *A. nidulans* is shown in Table 7; the hyphae were grown on defined medium containing a low concentration of glucose to ensure that a relatively sparse mycelium was formed in which it was unlikely that the rate of supply of oxygen would limit growth. The mean distance between adjacent nuclei apparently increased with distance from the tip; this type of nuclear distribution was observed in compartments ranging from 237 to $647 \mu\text{m}$ in length. In addition there was usually a considerable distance between the last nucleus in an apical compartment and the septum. Vacuolation of the cytoplasm also increased in the apical compartment with distance from the tip and was particularly marked at the rear of the compartment just before septation.

DISCUSSION

Synchronous mitosis has previously been observed during the early stages of growth of *Aspergillus nidulans* from spores (Rosenberger & Kessel, 1967; Figs 2 and 3) and in apical compartments of leading hyphae of *Alternaria solani* (King & Alexander, 1969) and *A. nidulans* (Clutterbuck, 1970). Mitosis in leading hyphae of both species is followed after a short interval by septation. These observations and the present results suggest the presence of a duplication cycle in apical compartments of leading hyphae which is analogous to the cell cycle of uninucleate cells. The main events of this duplication cycle are as follows. (1) Septation reduces the apical compartment to about half its maximum length. (2) The newly-formed apical compartment continues to increase in length at a linear rate. (3) The volume of cytoplasm per nucleus increases until at a critical ratio the nuclei are induced to divide more or less synchronously (synchronous mitosis in a germ-tube hypha is induced by a similar regulatory mechanism). (4) Mitosis is followed by septation which is completed when the apical compartment is about twice its original length.

This study has shown that, as predicted, the duration of the duplication cycle (given by the interval between successive cycles of septation) is approximately the same as the organism's doubling time (Figs 5 and 6; Table 4).

The mean volume of cytoplasm per nucleus in undifferentiated diploid mycelia was about 2.5 times that in haploid mycelia. Thus, as observed by Clutterbuck (1969), the volume of cytoplasm per genome is the critical ratio which regulates initiation of mitosis. In undifferentiated mycelia, mitosis probably occurs in intercalary compartments and their branch initials (Fig. 5) when, as a result of branch growth, the volume of cytoplasm per nucleus attains a critical value. Subsequently a branch follows a similar developmental pattern to a germ-tube hypha (Figs 6*a* and 7), eventually forming an apical compartment which extends at a linear rate (Fig. 10) and undergoes the duplication cycle described for leading hyphae.

The results suggest that, as in *Physarum polycephalum* (Sachsenmaier, Remy & Plattner-Shobel, 1972), a factor may accumulate in the cytoplasm of a compartment during interphase which, at a critical concentration, triggers mitosis. If a cytoplasmic signal does initiate mitosis, the observation that mitosis in adjacent compartments is not always synchronized (King & Alexander, 1969; present results) suggests that, unlike in *P. polycephalum* and other slime moulds, there is incomplete cytoplasmic mixing in a mycelium. Incomplete

cytoplasmic mixing is probably an inevitable consequence of the polarized nature of hyphal growth.

The observed decay in synchrony in undifferentiated mycelia (Fig. 2) certainly appears to be correlated with septation. Thus, although intra-compartment mitosis is synchronized, inter-compartment mitosis is asynchronous. Consequently an undifferentiated mycelium eventually assumes the characteristics (Fig. 2) associated with balanced growth of an asynchronous population (Campbell, 1957), i.e. all extensive properties of the mycelium increase at the same rate (Table 2).

The precision with which apical compartments of leading hyphae are divided into two by septation (Table 4; Fig. 6*b*) suggests that septal initiation is a well regulated event. In uninucleate cells (e.g. *Schizosaccharomyces pombe*; Johnson, Yoo & Calleja, 1973), in compartments of monokaryotic mycelia (Robinow, 1963; Niederpruem & Jersild, 1972), and in compartments of dikaryotic mycelia (Niederpruem & Jersild, 1972), septa are formed on cytoplasmic sites previously occupied by dividing nuclei. However, although apical compartments of leading hyphae of *A. nidulans* contain about 50 nuclei they form a maximum of only six to nine septa after synchronous mitosis. Thus in this organism there is a temporal (Clutterbuck, 1970) but not a quantitative or spatial relationship between mitosis and septation. King & Alexander (1969) suggest that nuclear concentration may partially determine the location of septa in apical compartments of *Alternaria solani* (see Table 7).

The mean interval between successive periods of septation (i.e. the duration of successive duplication cycles) in germ-tube hyphae is similar to that observed in leading hyphae and to the organism's doubling time (Fig. 6*a*; Table 4). However, septation divides the apical compartments of germ-tube hyphae unequally (Fig. 6*a*; Table 4). Leading hyphae, unlike germ-tube hyphae (Fig. 10), extend at a linear rate. Thus, although the frequency of septation is determined by the duration of the duplication cycle, sites of septation may in some way be influenced by the previous rate of extension of the hypha. Table 4 shows that the doubling time of a mycelium can be determined from the interval between successive septation cycles in leading hyphae at the margin of colonies. Bainbridge (1976) comes to the same conclusion.

Branching is an event which is physiologically analogous to cell division. The present results indicate a relationship between septation and branch initiation. However, branching can occur in the absence of septation (Table 5; Morris, 1975). Wall extension in fungi always appears to be associated with vesicles fusing with the existing wall; these vesicles may be generated throughout the cytoplasm of the mycelium (Collinge & Trinci, 1974). Vesicles may accumulate behind septa, initiating branches when their concentration exceeds a critical value. Since septa have pores there will be a balance between retention of vesicles in a compartment and their transport (Trinci & Collinge, 1973) to the next compartment.

There is clearly a mechanism which regulates the distribution of nuclei within the cytoplasm of mycelia (Fig. 4). As an apical compartment extends there is an increase in the mean distances between adjacent nuclei and between the tip and the first nucleus.

Trinci (1971) has shown that the rate of extension (K_r) of a hypha is a function of the length of its peripheral growth zone (w) and the organism's specific growth rate (α). Thus

$$K_r = w\alpha \quad (2)$$

Acceleration in the extension rates of germ-tube and branch hyphae (Fig. 10) appears to be correlated with increases in the length of the apical compartment (Fig. 7). Equation (2) indicates that the peripheral growth zone of such hypha must also increase in length. It is probably significant that germ-tube hyphae attain their maximum rate of extension (Fig.

10) at approximately the same time as their apical compartments attain their maximum length (Fig. 7).

The estimates of the length of the peripheral growth zone of leading hyphae of *A. nidulans* (330 to 370, 400 and 600 μm) suggest that the length of this zone is the same or approaches the maximum length attained by their apical compartments ($470 \pm 36 \mu\text{m}$). Clutterbuck & Roper (1966), Lhoas (1968), Nishi, Yanagita & Maruyama (1968) and Bainbridge (1976) have come to similar conclusions. The observation that hyphae continue to extend at a linear rate after septation, i.e. after reduction of the apical compartments to their minimum lengths, suggests that newly-formed intercalary compartments may for a time partly support the extension of leading hyphae, i.e. form part of their peripheral growth zones. The observation of King & Alexander (1969) of apical movement of cytoplasm through newly-formed but not older septa in leading hyphae of *Alternaria solani* supports this conclusion as does the wide variation in lag observed between septation and branch initiation in *A. nidulans*; in *G. candidum*, which forms complete septa, the lag between septation and branch initiation is much more constant (Fiddy & Trinci, 1976).

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REFERENCES

- BAINBRIDGE, B. W. (1976). Estimation of the generation time and peripheral growth zone of *Aspergillus nidulans* and *Alternaria solani* hyphae from radial growth rates and ranges of apical cell length. *Journal of General Microbiology* **97**, 125–127.
- BULL, A. T. & TRINCI, A. P. J. (1976). The physiology and metabolic control of fungal growth. *Advances in Microbial Physiology* (in the Press).
- CAMPBELL, A. (1957). Synchronization of cell division. *Bacteriological Reviews* **21**, 253–272.
- CLUTTERBUCK, A. J. (1969). Cell volume per nucleus in haploid and diploid strains of *Aspergillus nidulans*. *Journal of General Microbiology* **55**, 291–300.
- CLUTTERBUCK, A. J. (1970). Synchronous nuclear division and septation in *Aspergillus nidulans*. *Journal of General Microbiology* **60**, 133–135.
- CLUTTERBUCK, A. J. & ROPER, J. A. (1966). A direct determination of nuclear distribution in heterokaryons of *Aspergillus nidulans*. *Genetical Research, Cambridge* **7**, 185–194.
- COLE, G. T. & KENDRICK, W. B. (1968). A thin culture chamber for time lapse photomicrography of fungi at high magnifications. *Mycologia* **60**, 340–342.
- COLLINGE, A. J. & TRINCI, A. P. J. (1974). Hyphal tips of wild-type and spreading colonial mutants of *Neurospora crassa*. *Archives of Microbiology* **99**, 353–368.
- FENCL, Z., MACHEK, F. & NOVAK, M. (1969). Kinetics of product formation in multistage continuous culture. In *Fermentation Advances*, p. 301. Edited by D. Perlman. New York and London: Academic Press.
- FIDDY, C. & TRINCI, A. P. J. (1976). Nuclei, septation, branching and growth of *Geotrichum candidum*. *Journal of General Microbiology* **97**, 185–192.
- GOODAY, G. W. (1971). An autoradiographic study of hyphal growth of some fungi. *Journal of General Microbiology* **67**, 125–133.
- GROVE, S. W. & BRACKER, C. E. (1970). Protoplasmic organization of hyphal tips among fungi: vesicles and spitzenkörper. *Journal of Bacteriology* **104**, 989–1009.
- JOHNSON, B. J., YOO, B. Y. & CALLEJA, G. B. (1973). Cell division in yeasts: movement of organelles associated with cell plate growth of *Schizosaccharomyces pombe*. *Journal of Bacteriology* **115**, 358–366.
- KING, S. B. & ALEXANDER, L. J. (1969). Nuclear behaviour, septation and hyphal growth of *Alternaria solani*. *American Journal of Botany* **56**, 249–253.
- LHOAS, P. (1968). Growth rate haploidization of *Aspergillus niger* in medium containing *p*-fluorophenylalanine. *Genetical Research, Cambridge* **12**, 305–315.
- MITCHISON, J. M. (1971). *The Biology of the Cell Cycle*. Cambridge: Cambridge University Press.
- MORRIS, N. R. (1975). Mitotic mutants of *Aspergillus nidulans*. *Genetical Research, Cambridge* **26**, 237–254.
- NIEDERPRUEM, D. J. & JERSILD, R. A. (1972). Cellular aspects of morphogenesis in the mushroom *Schizophyllum commune*. *Critical Reviews in Microbiology* **545–576**.
- NISHI, A., YANAGITA, T. & MARUYAMA, Y. (1968). Cellular events occurring in growing hyphae of *Aspergillus oryzae* as studied by autoradiography. *Journal of General and Applied Microbiology* **14**, 171–182.

- ROBINOW, C. F. (1963). Observations on cell growth, mitosis, and division in the fungus *Basidiobolus ranarum*. *Journal of Cell Biology* **17**, 123-152.
- ROSENBERGER, R. F. & KESSEL, M. (1967). Synchrony of nuclear replication in individual hyphae of *Aspergillus nidulans*. *Journal of Bacteriology* **94**, 1464-1469.
- SACHSENMAIER, W., REMY, V. & PLATTNER-SHOBEL, R. (1972). Initiation of synchronous mitosis in *Physarum polycephalum*. A model of the control of cell division in eukaryotes. *Experimental Cell Research* **73**, 41-48.
- STEELE, G. C. & TRINCI, A. P. J. (1975). Morphology and growth kinetics of hyphae of differentiated and undifferentiated mycelia of *Neurospora crassa*. *Journal of General Microbiology* **91**, 362-368.
- TRINCI, A. P. J. (1971). Influence of the width of the peripheral growth zone on the radial growth rate of fungal colonies on solid media. *Journal of General Microbiology* **67**, 325-344.
- TRINCI, A. P. J. (1973). The hyphal growth unit of wild-type and spreading colonial mutants of *Neurospora crassa*. *Archiv für Mikrobiologie* **91**, 127-136.
- TRINCI, A. P. J. (1974). A study of the kinetics of hyphal extension and branch initiation of fungal mycelia. *Journal of General Microbiology* **81**, 225-236.
- TRINCI, A. P. J. & COLLINGE, A. J. (1973). Structure and plugging of septa of wild-type and spreading colonial mutants of *Neurospora crassa*. *Archiv für Mikrobiologie* **91**, 355-364.