SHORT COMMUNICATIONS

Synchronous Nuclear Division and Septation in Aspergillus nidulans

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(Accepted for publication 1 October 1969)

In the uninucleate cells of higher organisms, mitosis and cell division are coordinated both in time and position. In bacteria, similarly, although nuclear division is not an observable event, septation normally occurs only between pairs of nuclear bodies and at a fixed time in relation to DNA synthesis (Cooper & Helmstetter, 1968). In cœnocytes such as fungal hyphae it is less obvious that there is any special relationship between nuclear and cell division; however, King & Alexander (1969) have recently found that in Alternaria solani the hyphal tip cell is divided up by a number of septa after a wave of nuclear divisions has passed from the tip to the rear of the cell. Rosenberger & Kessel (1967) have shown that nuclear division is also synchronous in spore germlings of Aspergillus nidulans if they are grown on rich medium. The results presented here extend this observation to the longer tip cells of mature hyphae and show a pattern of nuclear division and septation very similar to that in Alternaria and Fusarium (Koenig & Howard, 1962).

Observations were made using a culture chamber consisting of a plastic Petri dish with a coverslip laid across a window cut in the base. The coverslip was inoculated with hyphae of a biotin requiring strain of Aspergillus nidulans from the Glasgow stocks (Pontecorvo et al. 1953), and was then covered with a permeable cellophane sheet (grade PT400, British Cellophane Ltd.) on top of which was poured a 3 to 4 mm. layer of complete medium agar (see Clutterbuck, 1969b, for formula). Observations of the hyphae sandwiched between the coverslip and the cellophane were made at 16 to 18° with a Reichert MeF phase-contrast microscope. At this temperature the growth of the hyphae is slow: about 50 μm. per hr (see Fig. 1).

Near the hyphal tips nuclei were visible as less dense areas of the cytoplasm each containing a dense nucleolus, but the nuclear membrane was indistinct. In the central region of the cell, nuclei were usually obscured by vacuoles, etc., but at the rear of the cell there was often a region of less dense cytoplasm in which spherical nuclei with clearly defined nuclear membranes and nucleoli could be seen.

As reported by Rees & Jinks (1952) and King & Alexander (1969), nuclear division was seen first at the hyphal tip and passed in a wave from there to the rear of the cell. During nuclear division, first the nucleolus and then the nuclear membrane became indistinct at a time when the nucleus was showing only slight signs of elongation. No details could then be made out until the smaller daughter nuclei became recognizable approximately 10 min. later. The wave of nuclear divisions took about 20 min. to pass from one end of the cell to the other: a distance of 440 to 700 μm. This length
can be calculated (Clutterbuck, 1969a) to contain 60 to 100 nuclei. In the branched hypha shown in the lower part of Fig. 1 the wave of nuclear division started at the tip of branch 'a' 13 min. after the start in the main hypha; in both cases the wave passed only backwards (branch 'b' was not examined at this time). In two cases it was observed that divisions occurred in two separate waves, the first at the tip and the second, somewhat later, at the rear of the cell. This may be taken as the first sign of the breakdown of synchrony that Rosenberger & Kessel (1967) found was the rule on poorer medium.

Fig. 1. The timing of nuclear division and septation in *Aspergillus nidulans*. The graphs show the growth rates of hyphal tips: the upper graph and indicators of time of nuclear division (ND) and time of formation of numbered septa refer to the hypha shown diagrammatically at the left, while the lower graph, etc. refer to the hypha at the right.

As can be seen from Fig. 1, the wave of nuclear division was followed after an interval of 20 to 40 min. by the formation of a series of septa at the rear of the tip cell which reduced its length by about half. This sequence of events has now been observed on ten occasions. The septa do not appear to form in any fixed order, although the tipmost one is often the first. The septa grow centripetally and are first seen as blebs on the side walls. On three occasions it has been noticed that two blebs were formed opposite the normal single one; one member of the pair then regressed while the other grew to form the septum. This suggests either that a septum may be initiated from more than one point in the same region or, more likely, that a septum is initiated at one point and spreads round the walls to form an annulus, or in the aberrant cases, a helix. Apart from the frequent formation of a septum at the base of a branch, there
is no indication what determines the sites of septum formation. There is no obvious correlation with the positions of nuclei, although in some tip cells where the nuclei are relatively sparse at the rear end, the septa divide this region into longer units than usual.

Figure 1 also shows that on this medium the hyphal tip may branch to give rise to a lateral or, less frequently, divide into two equal branches. As a rule the formation of a lateral branch does not affect the growth rate of the main hypha, but the branch itself generally takes some time to attain the growth rate of its parent. Where two more or less equal branches are formed the growth rate of both is reduced. Branches appear to arise with equal frequency at all stages of the nuclear division and septation cycle described here.

It is interesting to note that in the sterigmata, which are uninucleate cells of the conidial apparatus, cross-walls are formed in the normal way between pairs of daughter nuclei after nuclear division. The formation of coenocytes can therefore be regarded as due to the suppression of the majority of septa although a relationship between the time of nuclear division and septation is maintained.

I am grateful to Mrs June Baxendale for expert technical assistance.

REFERENCES


