SHORT COMMUNICATION

Vacuolation, Branch Production and Linear Growth of Germ Tubes of Candida albicans

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During germination of yeast cells of Candida albicans in liquid or solid serum-containing media the parent yeast cell and then sub-apical regions of the emerging germ tube became extensively vacuolated. Intercalary compartments were often almost entirely vacuolated, while the apices of germ tubes and branches maintained a high cell solids content. This length of non-vacuolate hypha may correspond to the growth zone of the organism. These observations may explain the observed unexpected linear growth of these germ tubes and the delay between septation and branch formation during filamentous growth.

INTRODUCTION

When yeast cells of Candida albicans are inoculated in serum-containing media they germinate to form branching mycelia which closely resemble those of other filamentous fungi but which exhibit two unusual features: (a) germ tubes extend at a linear rate and (b) there is a delay between septation and the production of a branch behind the septum (Gow & Gooday, 1982). In the filamentous moulds, germination is characterized by an exponential extension of the germ tube from the fungal spore (Trinci, 1971) and septation is normally closely followed by the formation of a branch behind the septum (Trinci, 1979).

Actively growing regions of hyphae maintain a high cell solids content and contain few vacuoles, while vacuoles are common in those areas of growth stasis or senescence that are found in older regions of fungal colonies (Park & Robinson, 1966; Barer & Joseph, 1955). This paper describes an early onset of vacuolation within germlings of C. albicans and suggests how this might account for the unusual growth patterns described for the organism.

METHODS

Organism and culture media. Candida albicans (Robin) Berkhout strain 3153 was from the London Mycological Laboratory. The media containing 20% (v/v) serum were prepared as described by Gow & Gooday (1982).

Growth in liquid and solid media. Yeast cell suspensions were prepared by washing cells from slopes of Sabouraud dextrose agar after 16 h growth at 37 °C. Liquid cultures were inoculated with 10⁶ yeast cells per ml 20% serum and incubated at 37 °C with shaking. Slide cultures on serum agar were prepared as described by Gow & Gooday (1982).

Microscopy. Liquid culture samples were centrifuged gently to remove serum and the germlings resuspended in an immersion medium of a concentrated solution of BSA (fraction V; Sigma) with a refractive index of 1.369. The solids content of this solution, obtained from a calibration curve (Ross, 1967), was 19.5% (w/v). Samples were examined by phase-contrast microscopy. Colonies growing on serum agar slabs were observed under bright-field illumination and photographed at intervals. All measurements are quoted as mean values ± σt (P = 0.05).

RESULTS

When ungerminated yeast cells were mounted in BSA immersion medium (Fig. 1a) they appeared phase dark and contained only small inclusions. When germ tubes had formed and...
Fig. 1. Germination of C. albicans in liquid (a–g) and on solid (h–m) serum-containing media. Germ-
lings were mounted in protein immersion medium and viewed under phase contrast (a–g) after 0 h (a),
2 h (b, c) and 4 h (d–g) incubation. Appearance of a developing colony under bright-field at 0 h (h) and
after 1 h (i), 1.5 h (j), 2.5 h (k), 4 h (l) and 4.5 h (m). Germination occurred at about 20 min. All
photographs are at the same magnification; the bar marker represents 20 μm.

elongated, each parent yeast cell developed a large refractile inclusion of low solid content (Fig.
1 b, c), presumably a vacuole. In longer germ tubes, vacuoles were seen within the parent cell and
the germ tube (Fig. 1 d, e, g), and phase-dark septa occurred at intervals along the germ tubes
(Fig. 1 c–g). Intercalary compartments often contained only small central phase-dark areas (Fig.
1 d, e, g), which may represent material around nuclei, since a nucleus occurs singly and centrally
in each intercalary compartment (Gow & Gooday, 1982). The apical regions of all germ tubes
were phase dark over an average length of 29.3 ± 3 µm (17 observations) suggesting the presence of a sub-apical slug of cytoplasm within the tip. After 4 h incubation the mother cell of some specimens had become phase dark, indicating some regeneration of cell solids (Fig. 1f). In other germlings second germ tubes were present (Fig. 1g).

Specimens grown on solid media showed the same features as above. Fig. 1(h–m) illustrates the development of a single germling. Initially the parent yeast cell appeared relatively dark with bright-field illumination (Fig. 1h). As germination proceeded light areas developed within the yeast cell (Fig. 1i) until, after 1.5 h, with a germ tube length of 26 µm, the parent cell interior was almost entirely light (Fig. 1j). Estimated volumes for the parent yeast cell (Fig. 1h) and the 26 µm germ tube (Fig. 1j) were 95 µm³ and 140 µm³, respectively, suggesting that most of the material within the germ tube derives from the parent cell. The germ tube apex remained dark throughout, and after 3 h growth a vacuolated intercalary compartment was present which had a central darker area as before (Fig. 1k). Four hours after germination branches were present immediately behind the second and third septa (Fig. 1l). As the branches grew away from the parent hypha, a slug of cytoplasm seemed to move into the branch (Fig. 1l, m).

**DISCUSSION**

The results suggest that the cytoplasm supporting initial growth of the germ tube is, for the most part, derived from the parent yeast protoplasm. Unlike fungal spores these yeast cells are metabolically active at the time of inoculation and require no protein synthesis de novo to support the switch to the filamentous growth form (Manning & Mitchell, 1980). A preformed and metabolically active volume of protoplasm appears to migrate forward with the extending apex in a similar way to the growth of the fungus *Basidiobolus ranarum* (Robinow, 1963). Unlike the situation in *B. ranarum*, however, the formation of a septum does not isolate a volume of cytoplasm capable of forming a sub-septal branch. In *C. albicans*, the intercalary compartments that are almost entirely vacuolated may therefore have to first synthesize cytoplasmic components to support branch production. This may explain why there is an observed delay between septation and branch formation during filamentous growth.

Mathematical models predict that exponential extension occurs in any hyphal system with apical growth, since this type of system is autocatalytic, i.e. an increasing volume of cytoplasm and vesicles is generated to support a single region of biosynthesis at the hyphal apex (Prosser & Trinci, 1979). Germ tubes of *C. albicans* incorporate wall precursors at their tips (Braun & Calderone, 1978) and so it has been unclear why they should grow linearly. From our observations we suggest that growth of these germ tubes is supported by a constant volume of cytoplasm originating from the parent cell and the system is, therefore, not autocatalytic.

The average length of the darker apical regions which may correspond to the peripheral growth zone was 29 ± 3 µm. This value is less than that calculated from kinetic data by Gow & Gooday (1982) as 48 µm.

Vacuolation in other fungi has been shown to be responsive to autogenic changes in the environment mediated by the production of ageing hormones such as bikaverin (Park & Robinson, 1967; Cornforth et al., 1971). In freshly inoculated serum media there would be little opportunity for accumulation of staling products; vacuolation in *C. albicans* germlings is, therefore, more likely to be controlled by some intrinsic factor or to occur in response to some other environmental stimulus.

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**REFERENCES**


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