SHORT COMMUNICATION

Duplication Cycle in Filamentous Forms of *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae strain S288C/1 was grown in a glucose-limited chemostat. At the fastest growth rates filamentous forms constituted a small percentage of the total cell number and were presumed to arise from the failure of cells to undergo cell separation. The phenomenon seemed to be distinct from chain formation, dimorphism and pseudomycelial growth and showed extensive analogies with the duplication cycle described for the filamentous fungi.

INTRODUCTION

The cell division cycle of *Saccharomyces cerevisiae* can be divided into an unbudded and a budded phase, both of which can vary in duration with the population doubling time, τ (Lord & Wheals, 1980; Thompson & Wheals, 1980). By extrapolation from the relationship of the budded phase to τ in batch culture, it was predicted (Lord & Wheals, 1980) that at $\tau = 65$ min (a value unattainable in batch culture) there would be insufficient time for completion of the budded phase and secondary bud formation would occur, that is the appearance of a second bud on a parent cell before the first bud had separated. This has been seen in *Candida utilis* (Vrana, 1973) during hypertrophic growth in a two-stage fermenter. Using chemostat culture (Thompson & Wheals, 1980) we have found that at the fastest growth rates ($\tau \leq 80$ min) *S. cerevisiae* may show a related, but different, alteration to cell morphology, in which a second bud appears on a parent cell but subsequent cell separation does not occur.

METHODS

Organism, media and growth conditions. These have been described previously (Thompson & Wheals, 1980).

Enzyme treatment. Filamentous cells were treated with Zymolyase 5000 (Kirin Brewery, Takasaki, Gumma Pref., Japan) at a concentration of 0.04 mg Zymolyase per mg dry wt yeast for 2 to 3 h at 23 °C (10% of the concentration needed to form spheroplasts in the same time).

RESULTS AND DISCUSSION

Bud scar analyses and volume measurements showed that at all growth rates less than $\tau = 80$ min, normal asymmetrical division was seen amongst the whole population (Thompson & Wheals, 1980), with daughter cells being born smaller and having longer cycle times than the parents from which they arose. At doubling times less than 80 min, small, stable clumps of yeast cells were also found (Fig. 1*a*), the number of individual cells in these clumps constituting 1 to 2% of the population. The individual cells could not be separated from each other by mild sonication, which was routinely performed on all chemostat samples. The clumps could be broken up into separate cells with buds by gentle digestion with Zymolyase 5000 at concentrations that did not lyse the cells. This technique has been used previously to demonstrate that cells can be artificially separated once cytokinesis has occurred

Fig. 1. (a) Phase-contrast micrograph of filament-forming yeast cells. The bar marker represents $5\ \mu\text{m}$. (b) Drawing of (a) with genealogical age of the cells marked. There are 16 daughters (age 0), eight parents of age 1, four of age 2, two of age 3, and one each of age 4 and 5. Twenty-nine of the 32 cells have small buds.

(Hartwell, 1971). The tracing (Fig. 1b) shows that there are 16 daughters (age 0) and 16 parents of different genealogical ages, eight of age 1, four of age 2, two of age 3 and one each of ages 4 and 5. The clump also shows that there is a high degree of synchrony, all but three cells having small buds. Bud scar analysis showed that asymmetrical division was occurring for the bulk of the population (single cells) but symmetry was evident for the filaments (Fig. 1a). Filaments of four to eight cells usually showed signs of synchrony, but as the number of cells increased so did the lack of synchrony. Microscopic observation of filaments on agar-slide culture revealed that the filaments remained stable but budded off normal single cells. The enzyme digestion and sonication experiments strongly suggested that the cells had undergone cytokinesis and probably primary septum formation (Cabib, 1975) but had failed to complete cell separation.

This phenomenon seems to be distinct from the three other known causes of filament formation in yeasts. First, chain formation is well known to occur, particularly among brewing strains, in *S. cerevisiae*. However, it is found in all cells of the population and at all growth rates. Furthermore, the chains tend to break up during the stationary phase of the growth cycle, in contrast to the stable structures revealed here (which can nevertheless bud off individual cells). Secondly, dimorphism has been frequently observed among the yeast-like fungi (Stewart & Rogers, 1978) although not with *S. cerevisiae*. More importantly, observations on *Mucor rouxii* grown in chemostat culture (Bartnicki-Garcia, 1968) showed that slow growth rates favoured the filamentous form, and fast growth rates the yeast-like form, the opposite of what was seen here. Thirdly, the phenomenon does not seem to be pseudomycelial growth, since there was no marked elongation of the bud, characteristic of this type of development. The structures described in this paper are perhaps best regarded as true filamentous mycelia with complete septation, the individual 'compartments' being more cylindrical than the normally prolate spheroids of the individual cells.

The filaments do not seem to be formed by the same mechanism that causes secondary bud

formation, since in filaments cell separation is abolished, whereas in secondary bud formation it is merely delayed (Lord & Wheals, 1980). Although cytokinesis and cell separation are triggered by the sequential pathway of the cell division cycle, they are not an integral part of the cycle and a further cycle can be initiated before they are complete. This is in agreement with the results of Hartwell (1971) using cell division cycle mutants blocked in cytokinesis and cell separation.

It can be seen that all cells had their buds at the opposite pole to that of the birth scar, in contrast to the free individual cells (Thompson & Wheals, 1980). This filamentous mode of growth, away from the pole at which a cell was born, leads to an interesting comparison of the cell cycle of the normally unicellular yeast *S. cerevisiae* with the duplication cycle for filamentous moulds (Fiddy & Trinci, 1976; Trinci, 1978).

The duplication cycle has three principal features which can be summarized as: (i) the apical compartment is reduced to half its length by septation; (ii) the apical compartment increases in length at a linear rate; (iii) a critical nuclear concentration triggers a round of mitosis and septation to be completed after a doubling in size of the apical compartment. The last completed cycle of Fig. 1 (a) budded off cells age 0 from cells of variable age. The mean length of age 0 cells is 93% that of their parents, indicating approximately symmetrical septation and feature (i). An examination of the chain of cells of age 0 to age 5 shows them to be of comparable size indicating that the filament has extended linearly during six generations (feature ii). The trigger for initiation of events leading to mitosis and septation is traverse of start (Hartwell *et al.*, 1974), which is dependent, under balanced growth conditions, on the attainment of a ploidy-related critical cell size, i.e. nuclear concentration (Hartwell & Unger, 1977; Lorincz & Carter, 1979). This event occurs at or near birth in cells growing at fast rates (Lords & Wheals, 1980). In the present experiments completion of the cycle occurred at cytokinesis when the daughter cells had reached the same size as their parents, that is, after a doubling in cell size, demonstrating feature (iii).

In addition, a number of other features of septate mycelial growth (Trinci, 1979) are shown by these filaments. First, growth only occurs at the tips of a mycelium. The marginal increase in size of older cells compared with younger cells (Fig. 1a) reinforces the observation (Bayne-Jones & Adolph, 1932) that growth of budding yeasts largely occurs by growth of the bud ($\equiv$ tip of the mycelium). Secondly, there is an exponential increase in the number of tips, shown in Fig. 1 (b) by the perfectly symmetrical genealogical age distribution with 32 cells producing 32 tips (29 having just budded, 3 still to bud). Thirdly, growth in older compartments occurs from immediately behind the septa, as is shown in Fig. 1 (a) by the position of the growing bud in the *parent* cells (age ≥ 1) being always behind the septa of a previous budding cycle.

This filamentous mode of development of *S. cerevisiae* parallels the duplication cycle and other features of filamentous mould growth lending weight to the view that differences in yeast-like and mycelial growth may often be of degree rather than kind.

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