Candida albicans and Yarrowia lipolytica as alternative models for analysing budding patterns and germ tube formation in dimorphic fungi

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The site for bud selection and germ tube emission in two yeasts, Candida albicans and Yarrowia lipolytica, was analysed. Both dimorphic organisms display different patterns of budding, which also differ from those described for Saccharomyces cerevisiae. C. albicans, which is diploid and (until now) lacks a known sexual cycle, buds in an axial budding pattern. During the yeast–hypha transition induced by pH, serum, \( N \)-acetylglucosamine (GlcNAc) or temperature, germ tube emergence occurs at approximately 50% in a polar manner, while the other 50% of cells show non-polar germ tube emission. Y. lipolytica, in which most of the natural isolates are haploid and which has a well-characterized sexual cycle, buds with a polar budding pattern independently of the degree of ploidy. Germ tube emission during the yeast–hypha transition in both haploid and diploid cells generally occurs at the pole distal from the division site (bipolar). The addition of hydroxyurea (HU), an inhibitor of DNA synthesis, also produces different effects. In its presence, and therefore in the absence of DNA synthesis, the yeast–hypha transition is completely abolished in Y. lipolytica. By contrast, in C. albicans germ tube emission in the presence of HU is similar to that observed in control cultures for at least 90 min under induction conditions. These results demonstrate that, rather than a single developmental model, several models of development should be invoked to account for the processes involved in the morphological switch in yeasts (the yeast–hypha transition).

Keywords: Yarrowia lipolytica, Candida albicans, budding, yeast–hypha transition, hydroxyurea

INTRODUCTION

Polarized cell division and spatially ordered cell growth (polarized growth) occur in a wide variety of species (both unicellular and multicellular). One of the best models for exploring both polarized cell growth and polarized cell division is the unicellular yeast Saccharomyces cerevisiae (Amon, 1996; Gónczy & Hyman, 1996; Hunter & Plowman, 1997; Johnson, 1995; Kron, 1997; Kron & Gow, 1995; Roemer et al., 1996). S. cerevisiae cells grow in a polarized manner during vegetative growth by budding from different sites on the cell surface. In this case, bud sites are defined by some intracellular landmark and are determined by several genes whose products are responsible for producing the defined pattern of bud selection (Chant & Pringle, 1995; Chant et al., 1995; Lew & Reed, 1995; Yang et al., 1997). S. cerevisiae also polarizes growth towards an extracellular signal, i.e. the mating pheromones during conjugation (Jackson & Hartwell, 1990a, b; Segall, 1993; Chenevert et al., 1994). Finally, under starvation conditions (diploid cells grown on nitrogen-limiting medium and haploid cells growing on limited glucose) S. cerevisiae is able to carry out pseudohyphal growth (Scherr & Weaver, 1953; Gimeno et al., 1992, 1993; Roberts & Fink, 1994). The switch in S. cerevisiae...
from yeast to pseudohyphal growth is accompanied by changes in several cellular processes. One of these is bud site selection; a change from the bipolar to the unipolar mode results in linear filamentous chains of cells (Mösch & Fink, 1997). This pseudohyphal growth resembles the dimorphic transition (a reversible change in the growth mode) that occurs in many pathogenic fungi (Vanden Bossche et al., 1993). In dimorphic yeasts the yeast–hypha transition has been analysed using several approaches (Gow, 1994). Most of them are based on the assumption that the yeast–filament switch is controlled by the same morphogenetic pathways in all fungi despite differences in individual signals and in the shape of the filaments.

Two yeasts, Yarrowia lipolytica and Candida albicans, are currently being used in our laboratory as dimorphic models for analysing the yeast–hypha transition. Y. lipolytica is a heterothallic yeast, amenable to genetic analysis, in which most of the natural isolates are haploid (Barth & Gaillardin, 1996). We have developed a simple system for inducing the yeast–hypha transition, replacing the carbon source glucose by a simple system for inducing the yeast–hypha transition, with or without the addition of 4% bovine calf serum. Also, germ tube formation was induced with GlcNac (1%). The dimorphic transition was induced as described previously (Martinez et al., 1990). The yeast–hypha transition in Y. lipolytica was carried out by a modification of a previously described method (Rodriguez & Dominguez, 1984). Cells in early exponential phase were centrifuged and resuspended in MMWN [0.67% yeast nitrogen base without amino acids and ammonium sulphate (Difco), 1% glucose] for 12 h at 23 °C and pH 4.5 or 6.8, containing glucose as carbon source, with or without the addition of 4% bovine calf serum. Also, germ tube formation was induced with GlcNac (1%).

C. albicans is the most frequently isolated fungal pathogen in humans and its growth mode is determined by environmental conditions (high temperature, high ratio of CO2 to O2, serum, nutrient-poor media, etc.). C. albicans is diploid, and has no known sexual cycle (Odds, 1988; Edwards, 1990) and several pathways regulating its cell morphology have been described (Saporito-Irwin et al., 1995; Leberer et al., 1996; Braun & Johnson, 1997; Stoldt et al., 1997; Lo et al., 1997). However, data concerning the budding patterns in both yeasts are scanty. Chaffin (1984) reported that in C. albicans the buds emerge primarily at one pole of the mother cell when cells are grown in the yeast form at 28 °C and pH 7.4, while bud sites not adjacent to the previous ones are found in yeast cells growing at 37 °C and pH 4.5. The same work also describes that the selection of sites for germ tube formation seems random after the cells are grown to stationary phase at 28 °C. In a more recent paper, the isolation of CaRSR1, a gene analogous to the S. cerevisiae BUD1 gene, has been described (Yaar et al., 1997). CaRSR1 is required for normal bud site selection, germ tube emergence (Lee’s broth) and hyphal elongation. In this study, we show that C. albicans and Y. lipolytica display different budding patterns, which also differ from that described for S. cerevisiae. Furthermore, the behaviour of both yeasts also differs with respect to germ tube emission in the absence of DNA synthesis. Taken together, all our data suggest that several experimental models would be necessary if general conclusions are to be drawn about the developmental pathways involved in the yeast–hypha transition in fungi.

METHODS

Strains and media. The following strains of Candida albicans were used in this study: C. albicans ATCC 10261, C. albicans ATCC 26553, C. albicans SC3314, C. albicans CAI4 (Δura3::imm434/Δura3::imm434), C. albicans CAI4 (Δura3::imm434/Δura3::imm434), C. albicans CAI4 (Δade2::bisG/ade2::bisG Δura3::imm434/Δura3::imm434) (Fonzi & Irwin, 1993). Yarrowia lipolytica strains used were: W28 (MatA), E129 (MatA lys1-23 ura3-302 leu2-270 xpr2-322), E150 (MatB bis-1 ura3-302 leu2-270 xpr2-322) (Barth & Gaillardin, 1996), SA-1 (MatB), ADD1 (MatA/MatB + /his-1 lys1-23/+ ura3-302/ura3-302 leu2-270/leu2-270 xpr2-322/xpr2-322), ADD2 (MatA/MatB lys1-25/+ + ura3-18/+ + /his-1) and ADD3 (MatA/MatB lys1-13/+ + len2-35/+ + /glcnac-1) (our laboratory). Strains were maintained by periodic transfer to slants of YED medium (1% yeast extract, 1% glucose, 2% agar). Yeast and hyphal growth patterns in C. albicans were obtained in Lee’s medium (Lee et al., 1975), pH 4.5 or 6.8, containing glucose as carbon source, with or without the addition of 4% bovine calf serum. Also, germ tube formation was induced with GlcNac (1%). The dimorphic transition was induced as described previously (Martinez et al., 1990). The yeast–hypha transition in Y. lipolytica was carried out by a modification of a previously described method (Rodriguez & Dominguez, 1984). Cells in early exponential phase were centrifuged and resuspended in MMWN [0.67% yeast nitrogen base without amino acids and ammonium sulphate (Difco), 1% glucose] for 12 h at 2 × 10⁷ cells ml⁻¹. Cells were then resuspended at 10⁷ cells ml⁻¹ in MM [0.67% yeast nitrogen base without amino acids (Difco), 1% glucose or 1% GlcNac as carbon sources].

Flow cytometry and light microscopy. Cells were fixed in 70% ethanol and processed for flow cytometry (Lew et al., 1992) or with 4,6-diamidino-2-phenylindole (DAPI) for nuclear staining (Sherman et al., 1986). A Becton–Dickinson FACScan was used for flow cytometry. Cells were photographed using a phase-contrast Zeiss axiophot microscope equipped with a 35 mm camera using Ilford FP4 Plus film (125 ASA).

Scanning electron microscopy (SEM). Cells were harvested, washed in 0.1 M sodium phosphate buffer, pH 7.4, prefixed with glutaraldehyde (5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4) for 1 h, washed twice in buffer and placed in 1% osmium tetroxide for 1 h at 4 °C. The material was subsequently washed in distilled water and dehydrated in a graded acetone series. The dehydrated cells, immersed in absolute acetone, were mounted on specimen holders, air-dried, coated with gold in a SEM Coating System (Bio-Rad) and examined under a Zeiss DSM 940 scanning electron microscope.

RESULTS

Yeast growth mode and induction of the yeast–hypha transition

A systematic collection of samples of both yeasts (C. albicans and Y. lipolytica) and of all the strains described in Methods growing in the yeast form and during the yeast–hypha transition was carried out. To determine budding patterns, cells were observed by staining their bud scars with Calcofluor white (a fluorescent compound that binds the chitin ring at the mother–daughter junction) or by SEM.
Budding patterns and germ tube emission in dimorphic yeasts

Table 1. Distribution of bud scars in *C. albicans* and *Y. lipolytica*

Only cells with two or more clearly visible bud scars were scored.

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of cells counted</th>
<th>Cells with two or more bud scars on the same pole</th>
<th>Cells with bud scars on both poles</th>
<th>Undetermined</th>
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<tr>
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**Fig. 1.** (a and b) Budding pattern of *C. albicans* SC5314 growing in the yeast form (cultures in the exponential phase of growth) as observed by SEM. All buds are located on one pole, clearly indicating the axial budding pattern. Bars, 1 µm.

Bud site selection during the yeast mode of growth

Observations of Calcofluor-stained cells (not shown) and SEM observations of all the strains tested revealed that the first bud site in an ellipsoidal cell almost always (95% of the cells) formed close to or at the tip of the cell in both haploid and diploid strains of *Y. lipolytica* and in diploid strains of *C. albicans*. This bud site formation was similar to what has been reported for *S. cerevisiae*. When we examined cells with two or more bud scars, a different picture emerged. In *C. albicans*, which is a diploid organism (Odds, 1988), SEM clearly showed that most the new bud sites (93%, Table 1) were adjacent to the previous ones (axial pattern, Fig. 1a and b) in agreement with the results described by Chaffin (1984). In *Y. lipolytica* (whose cells are more ellipsoidal) haploid (MatA and MatB) or diploid (MatA/MatB) cells, the second bud was almost invariably (in more than 95% of the cells, Table 1) formed opposite the first bud site (bipolar pattern, Fig. 2a and b). In the case of *S. cerevisiae*, it has been described that haploid cells (a cells and z cells) and diploid cells that are homozygous at the mating type locus (a/a and z/z cells) bud axially, while diploid (a/z) cells show a different budding pattern. Diploid daughters bud opposite the previous bud site (distal budding) while diploid mother cells mainly bud bipolarily, i.e. either adjacent to or opposite the previous bud site (Madden *et al*., 1992). Thus, our results with *Y. lipolytica* and *C. albicans* show clear differences with those described for *S. cerevisiae*.

Bud site selection during the yeast–hypha transition

Cells from all the strains of *C. albicans* and *Y. lipolytica* studied here were transferred to media that induce hyphal development (see Methods). Fig. 3 shows the
behaviour of *C. albicans* ATCC 26555 2 h after induction of the yeast–hypha transition (again, the axial budding pattern of the yeast mode of growth is clearly visible; triangles). The percentage of cells forming germ tubes was higher than 90% (Fig. 3a). The orientation of germ tube emergence with respect to the bud scar(s) from yeast growth was examined. Germ tubes formed in positions lateral (approx. 50% i.e. not emerging from either of the poles but rather from the side of the cell) or opposite (50%) to the bud scars (Table 2) in agreement with the results described by Chaffin (1984). Germ tubes emerged in a non-adjacent pattern regardless of the mode of induction (serum, pH, temperature or GlcNac) or the strain used (some small differences between strains were observed, Table 2), indicating that this type of behaviour is general for *C. albicans*. Our results clearly show that changes in the selection site for germ tube emission occur during the dimorphic switch with respect to the yeast mode of growth. Whether specific gene products regulate this change remains to be
Table 2. Bud site selection during the yeast–hypha transition in C. albicans and Y. lipolytica

Only cells with at least one clearly visible bud scar in one pole were scored.

<table>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SA-1</td>
<td>200</td>
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<tr>
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<tr>
<td>ADD1</td>
<td>200</td>
<td>183</td>
<td>10</td>
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Fig. 4. Scanning electron micrographs showing the pattern of bud site selection of haploid and diploid Y. lipolytica cells during the yeast–hypha transition. Haploid cells of strain SA-1 (a) and diploid cells of strain ADD2 after 4 (b) and 6 h (c, d) in the presence of GlcNAc. (e) Diploid cells of strain ADD1 8 h after the induction of the yeast–hypha transition. Bud scars were always located on one pole of the cells and the germ tube emerged in the opposite position (polar) to the bud scars. Bars, 1 µm.

elucidated. Since we synchronized all our cultures by starvation (all the cells were in the G1 phase of the cell cycle, see below), we cannot exclude the possibility that the lateral-polar pattern of germ tube emission might have been due to the loss of certain specific signals during this prolonged starvation period. Another point
meriting comment is the fact that germ tube diameters were one-quarter of the diameter of the yeast cells considered as spheres.

However, with Y. lipolytica, a different result was obtained. The yeast–hypha transition almost always occurred in a bipolar fashion. Fig. 4 shows that in both haploid (Fig. 4a) and diploid cells (Fig. 4b and c) germ tube emission occurred on the pole opposite the previous bud scar. In cells with bud scars on both poles (Fig. 4d and e), germ tube emission generally occurred on one pole, although for the time being we are unable to specify whether tube formation is adjacent to or opposite the previous budding site. Thus, with respect to the budding pattern, the behaviour of Y. lipolytica growing in the yeast form and during the yeast–hypha transition (haploid and diploid cells) is always the same; buds are formed mostly in a bipolar way (Table 2).

To induce the dimorphic switch, we starved the cells over a prolonged period of time (see Methods). Again, all the cells were in the G1 phase of the cell cycle (see Fig. 7). Clearly, in Y. lipolytica the budding pattern was not affected by refeeding, suggesting that the division site is marked by a strong signal which always specifies the location of the new bud on the distal pole. The Y. lipolytica hypha diameter corresponds to 60–100% of that obtained with the yeast cell considered as a sphere (Fig. 4).

Cell synchrony and the effect of hydroxyurea on the yeast–hypha transition

In a previous study, we demonstrated that in asynchronous cultures of Y. lipolytica the inhibition of DNA synthesis by hydroxyurea (HU) treatment prevented the yeast–hypha transition (Rodríguez et al., 1990). To extend this result and to compare the behaviour of C. albicans and Y. lipolytica during the dimorphic switch, we obtained synchronous cultures of both yeast species (see Methods). Fig. 5 offers a FACS analysis of C. albicans germ tube emission. Fig. 5(a), lane 1, shows the DNA content of an exponentially growing culture (yeast form) of C. albicans SC5314. Two peaks corresponding to cells in the G1 and G2 phases of the cell cycle can clearly be seen. Lane 2 represents the DNA content after starvation. The culture is synchronized and all cells are in the G1 phase. To address the issue of whether HU treatment inhibits germ tube emission, the culture was divided into two aliquots (Fig. 5a, control) and 200 mM HU was added to one of them (Fig. 5b). In both cultures, germ tube development was induced by our four standard conditions (pH, temperature, GlcNAc or serum) and all of them produced the same result. A clear increase in fluorescence, indicating normal DNA replication, was observed in Fig. 5(a) up to 120 min (control culture). Longer incubation times did not produce reliable results by FACS owing to the size of the hypha (see Fig. 6). However, as expected, DNA replication did not occur in the presence of HU (Fig. 5b). At the same time, the percentage of cells forming germ tubes was monitored (Fig. 6). Fig. 6(a) (corresponding to Fig. 5a, lane 1) shows a typical asynchronous yeast culture in the exponential phase of growth; both budded and unbudded cells are present. Fig. 6(b) (corresponding to Fig. 5, lane 2) shows our synchronous culture. No budding cells were present, as expected for a culture in the G1 phase. Figs 6(c) and 6(d) show the behaviour of the control culture at 120 min after germ tube induction. Normal germ tube formation occurred in more than 90% of the cells and DAPI staining revealed normal DNA replication and the migration of nuclei through the germ tube.

In the culture carried out in the presence of HU, DNA synthesis did not occur, as demonstrated by DAPI staining (Fig. 6f and, in agreement with our FACS results, Fig. 5b), although more than 85% of cells were able to form germ tubes (Fig. 6e). Comparison of both cultures (Fig. 6c and e) clearly showed that hyphal growth was reduced in the presence of HU (this effect was even more evident at prolonged incubation times; not shown). However, these experiments did not show whether the smaller length of the hyphae was a
Budding patterns and germ tube emission in dimorphic yeasts

Fig. 6. Germ tube formation. C. albicans cells were grown (a) under conditions inducing the yeast phase of growth, (b) synchronized and (c–f) observed 120 min after transfer to conditions inducing germ tube formation under normal conditions (c) (d, DAPI staining) and in the presence of HU (e) (f, DAPI staining). Bars, 20 µm.

direct effect, due to the lack of DNA replication, or an indirect effect; RNA and protein synthesis are known to be inhibited after 2 h in the presence of HU (Rodríguez et al., 1990). Our results for C. albicans indicate that germ tube formation occurs in the absence of DNA synthesis in a similar way to the normal processes up to times of approximately 90 min.

Similar experiments were carried out with Y. lipolytica. Fig. 7(a), lane 1 (and Fig. 8a), shows the DNA content of an exponentially growing culture (yeast form of Y. lipolytica haploid strain SA-1), with the typical distribution of cells in the G1 and G2 phases. Cultures synchronized prior to the induction of germ tube emission, with all the cells in the G1 phase of the cell cycle, are represented in Fig. 7(a), lane 2 (an image of the culture is shown in Fig. 8b). A constant increase in the DNA content, indicating that all the cells have more than one nucleus, is evident (Fig. 7a, lanes 4 and 5) in the control culture, which also developed a normal yeast–hypha transition (Fig. 8c and d). DNA replication did not occur in the presence of HU (Fig. 7b); in this case the drug completely blocked the yeast–hypha transition (Fig. 8e). The observation that some cells were able to
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DNA content

<table>
<thead>
<tr>
<th>Cell no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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Fig. 7. Flow cytometry analysis of the DNA content of *Y. lipolytica* SA-1 during germ tube induction in the absence (a) and presence (b) of 50 mM HU. Lanes: 1, yeast cells growing exponentially (panel a only); 2, synchronized cells; 3–5, samples at 4 (3), 6 (4) and 8 h (5) after induction of the yeast–hypha transition. About 10000 cells were examined in each panel.

bud is in agreement with the results described for *S. cerevisiae* by Slater (1973) and Hartwell (1976), and very few of them had a more cylindrical shape (Fig. 8e).

DISCUSSION

Polarized cell division occurs in a wide variety of unicellular and multicellular organisms and the elucidation of the mechanisms controlling growth polarity is essential for understanding cell division and hypha formation in fungi. The budding yeast *S. cerevisiae* undergoes polarized cell growth and polarized cell division at specific times during its life cycle and during the developmental processes leading to pseudohyphal growth. *S. cerevisiae* cells select bud sites on the basis of one of two predetermined patterns: MATα and MATα cells bud in an axial pattern and MATα/MATα cells bud in a bipolar pattern. It has also been described that during long incubation times on rich solid media, haploid *S. cerevisiae* cells switch from an axial to a bipolar budding pattern and carry out a process termed

Fig. 8. Yeast–hypha transition. *Y. lipolytica* cells were grown (a) under conditions inducing the yeast phase of growth, (b) synchronized and (c–e) observed 6 (c) and 8 h (d) after being transferred to conditions inducing the yeast–hypha transition (normal conditions, see Methods) and after 8 h in the presence of 50 mM HU (e). Bars, 20 µm.
invasive growth (Roberts & Fink, 1994). All these processes are well documented in this model yeast (Madden et al., 1992; Chant & Pringle, 1995; Chant et al., 1995; Kron & Gow, 1995; Lew & Reed, 1995; Roemer et al., 1996; Yang et al., 1997) and in-depth studies on the role of the genes of the MAPK pathway in *S. cerevisiae* and *C. albicans* morphogenesis have been carried out (Roberts & Fink, 1994; Herskowitz, 1995; Schultz et al., 1995; Leberer et al., 1996; Lo et al., 1997; Madhani & Fink, 1998a, b). However, although many yeast species are dimorphic, the data about budding patterns and germ tube emission are scanty in non-*Saccharomyces* yeasts. Here we compare the budding patterns of *C. albicans* and *Y. lipolytica*, a dimorphic heterothallic yeast that is genetically closer to filamentous fungi than many other budding yeasts (Barns et al., 1991).

We analysed several strains of *C. albicans* and *Y. lipolytica* and in the latter case the behaviour of haploid and diploid strains also. Although some subtle differences among strains of different backgrounds and in the same strain under the different conditions of the yeast–hypha transition were observed, we believe that the major features of our descriptions can be generalized.

Regarding bud formation during the yeast mode of growth, in both *Y. lipolytica* and *C. albicans* the first bud generally appeared on one pole of the cell, as has been described for *S. cerevisiae* (Madden et al., 1992). Also, and in agreement with the results obtained for *S. cerevisiae*, no cases of overlapping bud sites (overlapping bud scars) have ever been observed in axially budding or bipolar budding cells (Chant & Pringle, 1995; Figs 1, 2, 3 and 4).

On comparing budding patterns, we observed that diploid *Y. lipolytica* cells clearly budded in a bipolar way (like *S. cerevisiae*), while *C. albicans* followed an axial budding pattern. Surprisingly, haploid cells of *Y. lipolytica* also budded with a bipolar pattern, again differing from the behaviour of *S. cerevisiae* haploid cells. Our findings indicate that at least the three developmental models shown in Fig. 9 are necessary to understand the budding patterns of vegetatively growing yeast cells.

The choice of the budding pattern in *S. cerevisiae* is controlled by the mating type locus (Chant et al., 1995; Amon, 1996; Madden et al., 1992). Possibly, the observed differences between *S. cerevisiae* and *C. albicans* can be explained in terms of the lack of a sexual cycle in the latter yeast, although this hypothesis cannot be applied to *Y. lipolytica* because its haploid and diploid strains are stable and its conjugation and sporulation processes (although with lower efficiency than in *S. cerevisiae*) have been described exhaustively (Ogrydziak et al., 1978; Barth & Gaillardin, 1996). Furthermore, the loci corresponding to both mating types have been isolated and sequenced (Kurischko et al., 1992, 1999).

Regarding germ tube emission, again, we observed similar types of behaviour for all the strains tested under

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**Fig. 9.** Budding patterns of vegetatively growing yeast cells of *S. cerevisiae* (a), *C. albicans* (b) and *Y. lipolytica* (c). Possible models for site selection of pseudohypha formation in *S. cerevisiae* (d), germ tube emission in *C. albicans* (e) and the yeast–hypha transition in *Y. lipolytica* (f).
all the conditions assayed. In *S. cerevisiae*, pseudohyphal growth requires a polar budding pattern (Gimeno et al., 1992) and hence haploid filament formation depends on a switch from an axial to a bipolar mode of bud site selection (Roberts & Fink, 1994). Our results are essentially in agreement with this kind of behaviour although we stress that since *Y. lipolytica* haploid and diploid cells show a bipolar budding pattern such a switch is not necessary. In *Y. lipolytica* the second bud site and germ tube emission always occur on the pole distal to the preceding bud scar, independently of starvation and refeeding. This implies that nutritionally starved cells do not lose any specific component involved in bipolar budding-pattern signalling. For the time being we can only suggest that either axial genes involved in bud site selection, such as BUD3, BUD4, BUD10, etc. (Chant et al., 1995; Roemer et al., 1996), are not functional in *Y. lipolytica*, or that the gene products involved in the bipolar pattern act in this yeast in a dominant way. Experiments to test such possibilities are currently under way. In *C. albicans*, germ tube emergence is preceded by a switch from an axial to a different mode (lateral or polar) of bud site selection, resembling the behaviour of *S. cerevisiae* haploid cells during both invasive and pseudohyphal growth (Roberts & Fink, 1994).

HU inhibited DNA synthesis in *C. albicans* and *Y. lipolytica* (Figs 5 and 7). However, the addition of HU to cultures undergoing the yeast–hypha transition produced a different effect in both yeast species: whereas in *C. albicans* germ tube emission occurs in an apparently normal way up to 90 min after the addition of HU (in agreement with the results described by Shepherd et al., 1980), in *Y. lipolytica* the morphogenetic switch is completely abolished (in agreement with our previous results with asynchronous cultures, Rodriguez et al., 1990). One possible interpretation of this latter result could be that some preformed mRNA molecules with a short half life would be degraded during the long incubation times (4–6 h) necessary for germ tube induction in synchronized cells of *Y. lipolytica* in comparison with the time required by *C. albicans* (1–2 h).

While it is clear that dimorphism in fungi is controlled by multiple signalling pathways and that some of these are conserved among distantly related fungi, this study and several others suggest that several different models should be used if we are to understand how fungal cells integrate the information from different pathways to effect the dimorphic switch.

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**REFERENCES**


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