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Video article

High frame-rate resolution of cell division during *Candida albicans* filamentation

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ABSTRACT

The commensal yeast, *Candida albicans*, is an opportunistic pathogen in humans and forms filaments called hyphae and pseudohyphae, in which cell division requires precise temporal and spatial control to produce mononuclear cell compartments. High-frame-rate live-cell imaging (1 frame/min) revealed that nuclear division did not occur across the septal plane. We detected the presence of nucleolar fragments that may be extrachromosomal molecules carrying the ribosomal RNA genes. Cells occasionally maintained multiple nucleoli, suggesting either polyploidy, multiple nuclei and/or aneuploidy of ChrR., while the migration pattern of sister nuclei differed between unbranched and branched hyphae. The presented movie challenges and extends previous concepts of *C. albicans* cell division.

1. Introduction

Candida albicans is a multimorphic fungus that lives as a commensal yeast in humans and produces invasive hyphae and pseudohyphae as an opportunistic pathogen in patient groups with underlying immune deficiencies. Pseudohyphae are elongated yeast cells that fail to undergo cell separation following cytokinesis, resulting in chains of cells with constrictions at the septa that form at the mother-bud evagination site. In contrast, septin rings in hyphae are deposited from the growing tip as it passes the future site of septation (the presumptum) (Sudbery, 2001). In addition, the Spitzenkörper, an apical 'body' of vesicles, appears as a bright Mlc1-GFP (Myosin light-chain 1) spot at the hyphal tip throughout the cell cycle (Crampin et al., 2005). Another distinctive feature of hyphae is the lack of constrictions in the cell wall. Although different culture conditions can enrich for one form over the other, the yeast and hyphal morphologies comprise two ends of a continuum and fungal lesions in infected solid organ tissues invariably contain both cell types (Merson-Davies and Odds, 1989).

In contrast to most multinuclear filamentous fungi that undergo organellar streaming through open septal pores, *C. albicans* hyphae are compartmentalized by septa containing a 25 nm micropore that inhibits such traffic (Gow et al., 1980). *C. albicans* therefore requires tight regulation of nuclear division to ensure that each daughter cell contains a single nucleus prior to septum closure. Nonetheless, unusual mitotic divisions can give rise to *C. albicans* tetraploids, aneuploids or haploids (Suzuki et al., 1986; Rustchenko-Bulgac and Howard, 1993; Hickman et al., 2013), especially after drug exposure (Selmecki et al., 2006; Harrison et al., 2014).

Here we used high frame-rate time-lapse microscopy to report new insights into the spatio-temporal sequence of cell-cycle events in wild-type *C. albicans* cells that challenge our previous understanding of this process.

2. Results and discussion

The movie presented shows the growth of constitutively polarized *C. albicans* cells after initial evagination of a germ tube from 4 mother yeast cells (Yeast Cells 1–4, Fig. 2A, Movie 1). Filamentous growth was induced in serum at 37 °C in a micro-fabricated chamber featuring parallel walls (Brand et al., 2008). Nucleoli were visualized by expression of ribonucleolar protein, Nop1, fused to YFP and were used as a proxy for nuclear localization, as shown by co-localization of Nop1 with histones inside the nuclear membrane in *C. albicans* hyphae (Finley and Berman, 2005). Septum formation was visualized using Cdc3, one of the 5 *C. albicans* septins that comprise the septin ring, fused to GFP. The contrasting spatial organization of the two marker proteins rendered them easily

Abbreviations: GFP, green fluorescent protein; YFP, yellow fluorescent protein; DIC, Differential Interference Contrast.

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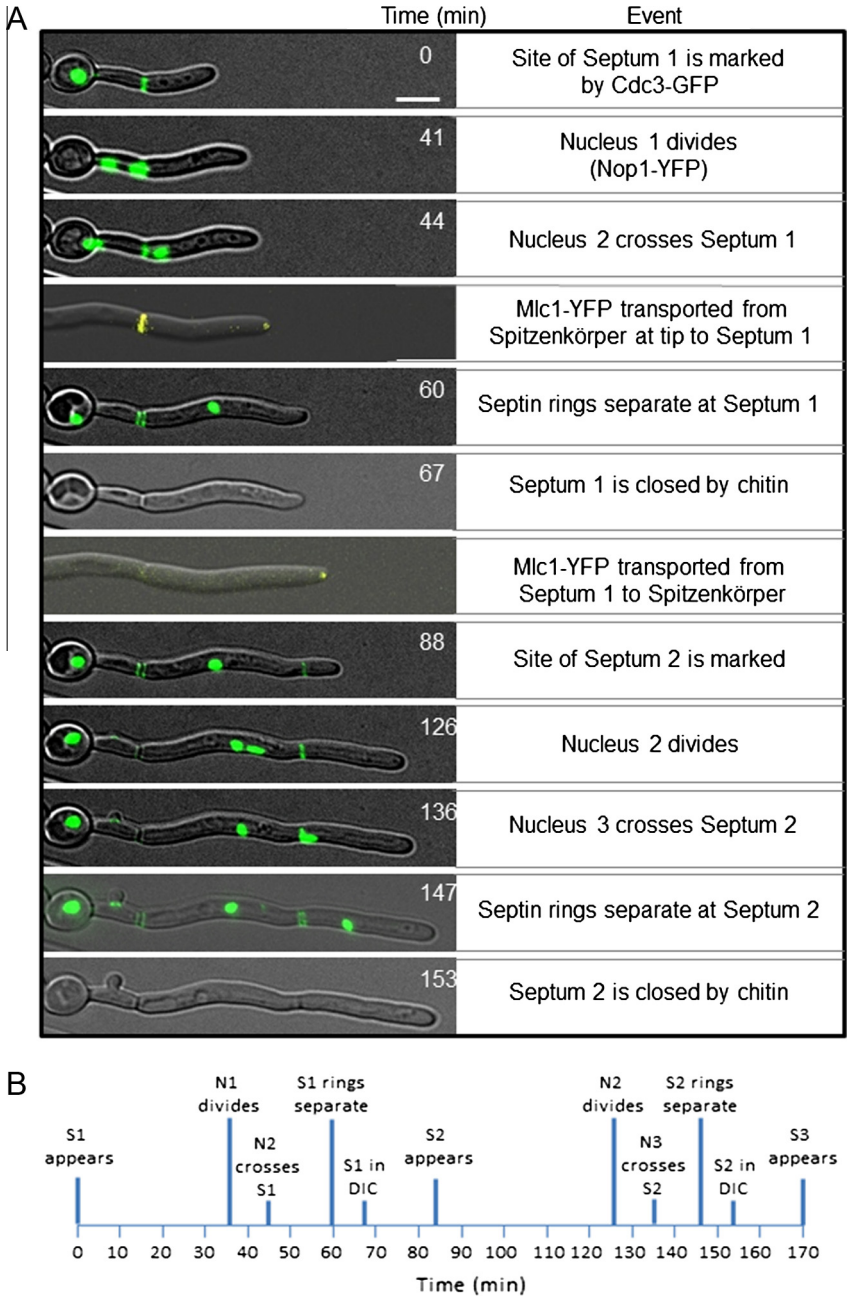

Fig. 1. Temporal and spatial sequence of cell-division events during polarized growth in *Candida albicans*. (A) The nucleolus and septin rings were visualized in double-tagged strain 8860 expressing Nop1-YFP and Cdc3-GFP as markers, respectively. In a separate strain, the Spitzenkörper was visualized using Mlc1-YFP. The timing of cell-cycle events was normalized across strains using the appearance of the closed septum, visible in DIC in both strains, as a shared reference point. Frame rate = 1 frame/min, bar = 5 μm. (B) Time course of cell-division events (194 events observed) (S, septum; N, nucleus; DIC, Differential Interference Contrast).

distinguishable in the same fluorescence channel (Finley and Berman, 2005).

2.1. Sequence of cell division events

During early hyphal growth, the Cdc3-GFP septins were observable as a spot within the hyphal tip until they marked the position of the first nascent septum by forming a single polymeric ring at the internal face of the plasma membrane. After mitosis and arrival of a sister nucleus in the daughter cell, the Cdc3-GFP signal at the presumptum separated into two distinguishable rings to make way for chitin deposition and formation of the septal wall. Closure of the septum by chitin deposition was observed using DIC microscopy. During septin ring separation, the Mlc1-YFP signal at the Spitzenkörper dimmed temporarily as a sub-population of Mlc1-YFP was seen streaming away from the Spitzenkörper to concentrate at the septum (Fig. 1A). After septal closure, the Mlc1-YFP signal at the septum was lost and the fluorescent signal returned to its previous intensity at the Spitzenkörper (Thomson et al., 2014). The observation that a subpopulation of Mlc1 can be temporarily relocalized to the septum suggests that the protein copy number within apical cells is relatively stable during growth, consistent with the idea that *C. albicans* hyphae grow using a constant volume of cytoplasm pushed forwards from the previous cell (Gow and Gooday, 1982). The sequence of cell division events and their relative timings are illustrated in Fig. 1A and B.