Trimorphic stepping stones pave the way to fungal virulence

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The fungal kingdom encompasses ≈1.5 million species (1) as diverse as single-celled yeasts, pathogens of animals/plants, and plant root symbionts. Fungi are eukaryotic, closely aligned with metazoans (2, 3). Animals and fungi diverged ≈1 billion years ago; their last common ancestor was unicellular, motile, and aquatic. Some fungi grow as unicellular yeasts, but most are filamentous multicellular organisms. Importantly, some fungi are dimorphic, growing as both yeast and filamentous forms (i.e., *Saccharomyces cerevisiae*). Yet others are trimorphic and grow as yeast, hyphae, and pseudohyphae (i.e., *Candida albicans*). *S. cerevisiae* pseudohyphal growth long eluded detection—it requires special conditions/strains and was lost during domestication (4). How pseudohyphae are related to yeast and hyphae (as a distinct fate or a continuum) was unknown until the report of Carlisle *et al.* (5) in this issue of PNAS. They reveal that pseudohyphae are intermediate between yeast and hyphae, with implications for pathogen–host interactions and fungal evolution.

C. albicans is a trimorphic fungus from the Ascomycota, diverged from *S. cerevisiae* ≈200 million years ago (6, 7), and a commensal of the human microbiota of the gastrointestinal tract, mucous membranes, and skin. *C. albicans* infects humans, causing oropharyngeal disease, vaginitis, and systemic life-threatening infection. Serum induces *C. albicans* yeast to produce germ tubes, and growth in defined media elicits two filamentous growth modes: pseudohyphae and hyphae (Fig. 1*A*). Pseudohyphae resemble hyphae, but are morphologically distinct. In both modes the normally ovoid yeast cells are elongated, but in pseudohyphae each cell–cell junction is constricted, and the diameter between cell walls is wider in the middle than the ends. In contrast, hyphal cell walls are parallel with no mother–daughter or septal junction constrictions. Pseudohyphae and hyphae also differ with respect to septin localization and nuclear division (7). Given these differences, it has been unclear whether pseudohyphae are an intermediate between yeast and hyphae or are an alternative fate (Fig. 1). This question, and implications for virulence, were addressed by Carlisle *et al.* with engineered strains (5).

The *C. albicans* dimorphic yeast–hyphae transition is thought to underlie its success as a pathogen. Mutants locked as yeasts (lacking Cph1 and Efg1 transcription factors or cyclin Hgc1) or filaments (lacking the Tup1 repressor) are both avirulent, linking both forms to pathogenesis (8–10). Subsequently, a strain was engineered in which morphogenesis is controlled by regulated expression of a filamentation repressor, Nrg1, with the *tet* promoter (11). Cells grown without doxycycline express Nrg1 and grow as yeast, whereas growth with doxycycline repressed Nrg1 and filamentous growth ensued. Animals infected with yeast remained healthy yet harbored a significant latent fungal burden in the kidney. Adding doxycycline to drinking water activated filamentation, with progression to lethal infection. These studies provide robust support for the concept that dimorphic transitions underlie *C. albicans* virulence, and they show that yeast can penetrate tissues, whereas hyphae are necessary for progression to lethal infection. In cultured macrophages *S. cerevisiae* is killed after phagocytosis, whereas *C. albicans* yeast switch to hyphae, killing and escaping macrophages (12).

The studies of Carlisle *et al.* (5) involve controlling expression of Ume6, a zinc-finger transcription factor downstream of Nrg1 in circuits for dimorphic transition and virulence (13, 14). In their studies, Ume6 was expressed from the doxycycline-regulatable promoter (*tet-UME6*), leading to controlled consequences: yeast with the promoter off (dox+), and hyphae in response to high-level Ume6 (dox−), even under non-filament-inducing conditions. Intermediate expression elicited a third fate: pseudohyphae. Increasing Ume6 levels converted pseudohyphae to hyphae, and hybrid hyphal/pseudohyphal filaments were observed. When the *tet-UME6* strain was grown as hyphae (dox−) and then shifted to repress (dox+), filaments produced a majority of pseudohyphae by 3 h and yeast by 7 h. Thus both filamentous modes can be reversibly evoked by expressing a single regulatory element in a dosage-dependent fashion, providing evidence that pseudohyphae are intermediate between yeast and hyphae, rather than a distinct fate. When animals were infected with yeast of the *tet-UME6* strain (dox−), increased Ume6 expression led to enhanced hyphal growth and tissue invasion and more rapid demise; animals given doxycycline survived much longer. Hence, hyphal growth (or hyphal-specific gene expression) promotes *C. albicans* virulence.

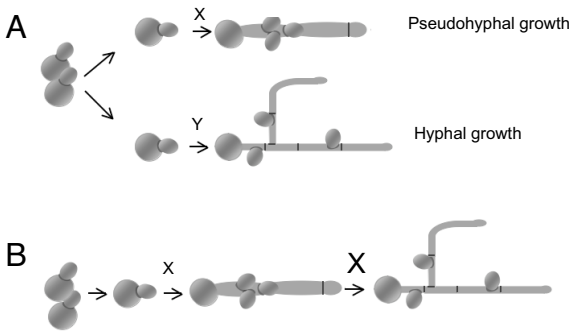

Fig. 1. Fungal morphological transitions. (*A*) Yeast undergoes dimorphic transitions to pseudohyphae or hyphae controlled by different elements (X, Y). (*B*) A continuous transition from yeast to pseudohyphae to hyphae controlled by a dosage-dependent factor (x, X).

The findings of Carlisle *et al.* (5) have broad implications for dimorphism in fungal pathogenesis (Fig. 2). *S. cerevisiae* and *C. albicans* both undergo yeast–pseudohyphal transitions, but only *C. albicans* develops hyphae. As *C. albicans* evolved into a successful commensal of the mammalian gastrointestinal tract, formation of hyphae in biofilms was likely necessary to compete with bacteria, or to form cooperative multispecies biofilms (15). Hyphae are also critical for *C. albicans* to survive and escape host macrophages. As a result, the trimorphic species *C. albicans* is a successful commensal and pathogen, whereas the dimorphic yeast *S.*

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Ascomycota				Basidiomycota	Zygomycota
Saccharomyces cerevisiae	*Ashbya gossypii*	*Candida albicans*	*Histoplasma capsulatum*	*Cryptococcus neoformans*	*Mucor circinelloides*
Saprobe	Plant pathogen	Human pathogens			
•Dimorphic •Yeast morphotype commonly observed during most growth conditions • Pseudohyphal growth observed during nitrogen limiting conditions	•Monomorphic • Filamentous fungus closely related to *S. cerevisiae*, grows exclusively as hyphae. •Causative agent of stigmatomycosis in cotton and citrus plants	•Trimorphic • Yeast phase important for dissemination within the host • Hyphal growth is essential for infection and colonization of host and for biofilm formation on catheter and mucosal surfaces	•Dimorphic • Yeast form responsible for human infection • Mycelium form commonly found in soil • Upon human infection, body temperature induces hyphae to yeast transition	•Trimorphic • Yeast form responsible for human infection •Sexual cycle leads to hyphal growth and production of infectious basidiospores	•Dimorphic •Filamentous mold. Yeast phase induced during low oxygen conditions • Inhaled conidia germinate into hyphal extensions in the host
✓ Yeast ✓ Pseudohyphae ✗ Hyphae	✗ Yeast ✗ Pseudohyphae ✓ Hyphae	✓ Yeast ✓ Pseudohyphae ✓ Hyphae	✓ Yeast ✗ Pseudohyphae ✓ Hyphae	✓ Yeast ✓ Pseudohyphae ✓ Hyphae	✓ Yeast ? Pseudohyphae ✓ Hyphae

Fig. 2. Evolution of fungal form and function. Mono-, di-, and trimorphic fungi are shown.

cerevisiae is an uncommon pathogen (16). Exploring genetic circuits controlling dimorphism in both species, and those between them, is likely to be illustrative.

The ability of fungi to infect humans occurred independently multiple times, and successful human pathogens are diverse (Fig. 2). The prominent role for hyphae in *C. albicans* virulence is contrasted with a leading role for yeast in other pathogens. The dimorphic fungal pathogens (i.e., *Histoplasma capsulatum*) are thermally dimorphic, growing as filamentous molds at lower environmental temperatures and as yeast at 37 °C (17). Humans are infected by inhaled conidia that must convert to yeast to be pathogenic. Strains or mutants locked as filaments are avirulent (18, 19). In the Basidiomycota phylum, the plant pathogen *Ustilago maydis* can grow as yeast, but successful plant infection requires filamentous dikaryons produced by mating (20). The human pathogen *Cryptococcus neoformans* undergoes a similar sexual dimorphic transition, but spores or dried yeast cells infect humans and only yeast occur in the host (21). Other fungi infecting plants and humans are strictly filamentous. How these diverse pathogenic strategies subvert host defenses may share common principles, or converge via distinct mechanisms.

The findings of Carlisle *et al.* (5) have broader evolutionary implications. The finding that pseudohyphae are a developmental way station between yeast and hyphae suggests stepwise evolution (Fig. 1*B*). We can envision an ancestral fungus as a unicellular yeast making the leap, first to pseudohyphae and then hyphae. This would have facilitated foraging for mating partners and nutrients and rapid colonization of habitats, including plants/animals. In this model mutations may block just hyphal growth, or hyphal and pseudohyphal growth. Overexpression of other regulatory components may enable dimorphic yeasts to produce hyphae. Most fungi are filamentous with no yeast form, thus mutations preventing the dimorphic return to yeast likely confer selective benefits. These studies may provide insights into how closely related species evolved divergent life styles (Fig. 2). *S. cerevisiae* grows as yeast and pseudohyphae but not hyphae, whereas its close relative *Ashbya gossypii* grows only as hyphae and *Holleya sinecauda* grows as yeast, pseudohyphae, and hyphae (22–24). *A. gossypii* thus lacks functions for hyphal–yeast transition, and *H. sinecauda* has factors enabling hyphal growth from yeast and pseudohyphae. Recent studies reveal that the pescadillo ortholog promotes production of budding yeast cells from *C. albicans* hyphae (25), and related factors may enable *Cryptococcus neoformans* hyphae to produce yeast cells (21). Other fungi, such as *Mucor* sp. in the Zygomycota, prominently grow as filamentous fungi in lab aerobic conditions yet also grow as multibudded yeasts in anaerobic/high-CO_2 conditions (26). Conditions or mutations enabling other filamentous fungi to grow as pseudohyphae or yeast may remain to be discovered. Understanding genetic circuits evoking transitions in fungal form, and impact on biology and infection, will continue to fascinate for years to come.

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1. James TY, *et al.* (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. *Nature* 443:818–822.
2. Baldouf SL, Palmer JD (1993) Animals and fungi are each other's closest relatives: Congruent evidence from multiple proteins. *Proc Natl Acad Sci USA* 90:11558–11562.
3. Wainright PO, *et al.* (1993) Monophyletic origins of the metazoa: An evolutionary link with fungi. *Science* 260:340–342.
4. Gimeno CJ, *et al.* (1992) Unipolar cell divisions in the yeast *Saccharomyces cerevisiae* lead to filamentous growth: regulation by starvation and *RAS*. *Cell* 68:1077–1090.
5. Carlisle PL, *et al.* (2009) Expression levels of a filament-specific transcriptional regulator are sufficient to determine *Candida albicans* morphology and virulence. *Proc Natl Acad Sci USA* 106:599–604.
6. Berman J, Sudbery PE (2002) *Candida albicans*: a molecular revolution built on lessons from budding yeast. *Nat Rev Genet* 3:918–930.
7. Sudbery P, *et al.* (2004) The distinct morphogenic states of *Candida albicans*. *Trends Microbiol* 12:317–324.
8. Lo HJ, *et al.* (1997) Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 90:939–949.
9. Zheng X, *et al.* (2004) Hgc1, a novel hypha-specific G1 cyclin-related protein regulates *Candida albicans* hyphal morphogenesis. *EMBO J* 23:1845–1856.
10. Braun BR, *et al.* (2000) Identification and characterization of TUP1-regulated genes in *Candida albicans*. *Genetics* 156:31–44.
11. Saville SP, *et al.* (2003) Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryotic Cell* 2:1053–1060.
12. Lorenz MC, Fink GR (2002) Life and death in a macrophage: role of the glyoxylate cycle in virulence. *Eukaryot Cell* 1:657–662.
13. Kadosh D, Johnson AD (2005) Induction of the *Candida albicans* filamentous growth program by relief of transcriptional repression: a genome-wide analysis. *Mol Biol Cell* 16:2903–2912.
14. Banerjee M, *et al.* (2008) UME6, a novel filament-specific regulator of *Candida albicans* hyphal extension and virulence. *Mol Biol Cell* 19:1354–1365.
15. Nobile CJ, Mitchell AP (2007) Microbial biofilms: e *pluribus unum*. *Curr Biol* 17:R349–353.
16. McCusker JH (2006) in *Molecular Principles of Fungal Pathogenesis*, eds Heitman J, Filler SG, Edwards JE, Mitchell AP (Am Soc Microbiol, Washington, DC), pp 245–259.
17. Klein BS, Tebbets B (2007) Dimorphism and virulence in fungi. *Curr Opin Microbiol* 10:314–319.
18. Maresca B, *et al.* (1977) Regulation of dimorphism in the pathogenic fungus *Histoplasma capsulatum*. *Nature* 266:447–448.
19. Nemecek JC, *et al.* (2006) Global control of dimorphism and virulence in fungi. *Science* 312:583–588.
20. Kamper J, *et al.* (2006) Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444:97–101.
21. Hull CM, Heitman J (2002) Genetics of *Cryptococcus neoformans*. *Annu Rev Genet* 36:557–615.
22. Wendland J, Walther A (2005) *Ashbya gossypii*: a model for fungal developmental biology. *Nat Rev Microbiol* 3:421–429.
23. Dietrich FS, *et al.* (2004) The *Ashbya gossypii* genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome. *Science* 304:304–307.
24. Gastmann S, *et al.* (2007) A molecular toolbox for manipulating *Eremothecium coryli*. *Microbiol Res* 162:299–307.
25. Shen J, Cowen LE, Griffin AM, Chan L, Köhler JR (2008) The *Candida albicans pescadillo* homolog is required for normal hypha-to-yeast morphogenesis and yeast proliferation. *Proc Natl Acad Sci USA* 105:20918–20923.
26. Bartnicki-Garcia S, Nickerson WJ (1962) Induction of yeast-like development in Mucor by carbon dioxide. *J Bacteriol* 84:829–840.