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Mechanisms of Cytokinesis in Basidiomycetous Yeasts

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Abstract

While mechanisms of cytokinesis exhibit considerable plasticity, it is difficult to precisely define the level of conservation of this essential part of cell division in fungi, as majority of our knowledge is based on ascomycetous yeasts. However, in the last decade more details have been uncovered regarding cytokinesis in the second largest fungal phylum, basidiomycetes, specifically in two yeasts, *Cryptococcus neoformans* and *Ustilago maydis*. Based on these findings, and current sequenced genomes, we summarize cytokinesis in basidiomycetous yeasts, indicating features that may be unique to this phylum, species-specific characteristics, as well as mechanisms that may be common to all eukaryotes.

Keywords

Cytokinesis; Basidiomycetes; yeast; septins; *Cryptococcus*; *Ustilago*

1. Introduction

Cytokinesis is the final step in the cell cycle that results in two physically separate cytoplasms of the dividing cells. The partition of cytoplasms is precisely coordinated with chromosomal segregation and subject to complex regulation, as its failure may result in aneuploidy (D'Avino, Giansanti et al. 2015). In animal cells, the major force that drives the ingression of the plasma membrane during cytokinesis is the constriction of the cortex-associated ring consisting of filamentous F-actin and non-muscle myosin referred to as the actomyosin ring (AMR), otherwise known as contractile actomyosin ring. In fungi, in addition to the constriction of the AMR, a new cell wall is synthesized between the dividing cells in the form of septa. In yeasts, the physical separation of the daughter cells is triggered by septum hydrolysis.

Although fungi and animals have diverged about one billion years ago, major cytokinesis events are relatively well conserved between the two kingdoms (Pollard 2010). In fact, largely what we know about animal cytokinesis has come from studying two classic models for eukaryotic biology, ascomycete yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (model yeasts) (Pollard 2010, Yanagida 2014). On the other

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hand, mechanisms of events that accompany the assembly and the constriction of the AMR exhibit considerable plasticity across fungi and animals (Balasubramanian, Bi et al. 2004, Balasubramanian, Srinivasan et al. 2012, Gu and Oliferenko 2015). For instance, budding yeasts belonging to the second largest fungal phylum, Basidiomycota, undergo nuclear division through mechanisms significantly different from those described for ascomycete yeasts (Poon and Day 1976, Mochizuki, Tanaka et al. 1987). Therefore, studying basidiomycetes may be critical for better understanding the evolution of the mechanisms of cytokinesis. While comparative genomics with other phyla may be informative, comparative cell biology of basidiomycete yeasts may provide functional insights beyond those obtained from studying model yeasts.

Ascomycota and Basidiomycota have diverged approximately 400 million years ago (Taylor and Berbee 2006). Basidiomycetes are characterized by the production of basidiospores, via sexual reproduction, usually in a dimorphic manner (Lee, Ni et al. 2010). Both the sexual dimorphic and asexual yeast forms are common in all three main classes of Basidiomycota (Agaricomycotina, Pucciniomycotina, Ustilaginomycotina) (Fell 2001, Morrow and Fraser 2009). Most known basidiomycete yeasts undergo division by budding, while fission has not been well characterized in this phylum, except for limited studies on the *Trichosporon* species (Gueho, Smith et al. 1992).

Here we summarize current literature about cytokinesis in basidiomycetous yeasts, and analyze sequenced genomes to address the following questions: Is cytokinesis significantly different in basidiomycetes as compared to ascomycetes? Could elucidating cytokinesis in basidiomycetes improve our understanding of the evolution and mechanisms of this essential part of cell division?

This review focuses on two basidiomycetes that have been investigated relatively extensively, a representative of Ustilaginomycotina, corn smut, *Ustilago maydis* and a member of Agaricomycotina, pathogen of humans *Cryptococcus neoformans*. *U. maydis* exhibits two major morphologies, saprophytic yeast and filamentous hyphae that propagate within the plant host during infection. The yeast divides by budding and the bud grows mostly by tip extension resulting in an elongated, cigar shape morphology (Banuett 1992, Steinberg and Perez-Martin 2008, Vollmeister, Schipper et al. 2012) (Figure 1). *C. neoformans* yeast cells divide by budding, including the apical to isotropic switch, which results in a characteristic round shape similar to that of *S. cerevisiae* (Kozubowski and Heitman 2012, Wang and Lin 2015) (Figure 1). In both *U. maydis* and *C. neoformans* the hyphae form as a result of mating. However, in *C. neoformans*, it is the yeast form that causes infection (Lin 2009). *U. maydis* and *C. neoformans* belong to distinct classes of Basidiomycota. Therefore, our knowledge about cytokinesis in these yeasts may reflect mechanisms that are common to all basidiomycetes as well as pathways that are class or species-specific.

Several excellent reviews have been published recently that describe cytokinesis in *S. cerevisiae* and *S. pombe* and we refer the reader to these great resources for more information (Weiss 2012, Wloka and Bi 2012, Willet, McDonald et al. 2015, Juanes and Piatti 2016, Meitinger and Palani 2016, Perez, Cortes et al. 2016, Rincon and Paoletti 2016).

Here we provide a brief overview of the mechanisms in both model yeasts and focus mostly on studies describing cytokinesis in basidiomycetous yeasts.

2. Mechanisms of entry into cytokinesis

The timing of cytokinesis has to be tightly controlled so that it always follows a successful chromosomal segregation. Mitotic cyclin-dependent kinase (Cdk) is the main regulator that prevents the onset of cytokinesis until the chromosomes are properly segregated and inactivation of Cdk allows the cytokinesis to proceed (Morgan 1999).

In *S. cerevisiae*, the entry to cytokinesis is regulated through the signaling dependent on the Polo kinase and a network of proteins collectively known as the Mitotic Exit Network (MEN) (Lee, Frenz et al. 2001, Lee, Jensen et al. 2001, Petronczki, Lenart et al. 2008, Meitinger, Palani et al. 2012). The MEN signaling initiates during late anaphase at the spindle pole body (SPB) where the GTPase, Tem1, stimulates the kinase, Cdc15, to activate the Mob1-Dbf2 kinase complex. Mob1-Dbf2 in turn promotes the release of the protein phosphatase, Cdc14, from the nucleolus. Cdc14 reverses phosphorylation of a number of Cdk1 targets leading to mitotic exit and cytokinesis (Jaspersen, Charles et al. 1998, Visintin, Craig et al. 1998) (Visintin, Craig et al. 1998, Shou, Seol et al. 1999, Visintin, Hwang et al. 1999, Meitinger, Palani et al. 2012, Miller, Hall et al. 2015). A subset of Cdc14 is also released from the nucleolus during early anaphase by another signaling cascade called FEAR (for Cdc fourteen **early** release), which is only transient and not sufficient to trigger cytokinesis but essential for timely exit from mitosis (Jensen, Geymonat et al. 2002, Stegmeier, Visintin et al. 2002). The MEN also plays a role in cytokinesis that is independent of mitotic exit and the release of Cdc14 from the nucleolus (Lippincott, Shannon et al. 2001, Hotz and Barral 2014).

In *S. pombe*, the Septation Initiation Network (SIN) is analogous to MEN (Chang, Morrell et al. 2001, Wachowicz, Chasapi et al. 2015, Willet, McDonald et al. 2015, Rincon and Paoletti 2016). The major players of SIN include the small G protein Spg1p; its GTPase-Activating Protein (GAP) Byr4p-Cdc16p; three protein kinases and their associated regulators Cdc7p, Sid1p-Cdc14p, and Sid2p-Mob1p; and two scaffolding factors Sid4p-Cdc11p. In contrast to MEN, the activation of SIN signaling is not dependent on the release of the Cdc14 homologue, Clp1, from the nucleolus (Bardin and Amon 2001). Although Clp1 accumulates in the nucleolus and at the SPBs in G1 and S phase, it gets released from the nucleolus in prophase and localizes to the medial ring and the mitotic spindle (Trautmann, Wolfe et al. 2001). Continual localization of Clp1 outside of nucleolus is maintained based on SIN signaling, which is necessary for the cytokinesis checkpoint (Cueille, Salimova et al. 2001, Mishra, Karagiannis et al. 2005).

How cytokinesis is activated in basidiomycetous yeasts is presently unknown. Both *U. maydis* and *C. neoformans* exhibit several features of nuclear division that appear strikingly different from ascomycete budding yeasts (Figure 1). The nuclear envelope (NE) partially breaks open during mitosis (Theisen, Straube et al. 2008, Kozubowski, Yadav et al. 2013). Prior to metaphase the entire chromatin translocates to the daughter cell where the spindle is formed and segregation of chromosomes proceeds in the direction from the bud into the

mother cell (McLaughlin 1984, Theisen, Straube et al. 2008, Kozubowski, Yadav et al. 2013). Strikingly, in *C. neoformans* the nucleolus disintegrates prior to metaphase (Kozubowski, Yadav et al. 2013), a process that may be similar in *U. maydis* based on studies performed in a more distantly related species *Microbotryum violaceum* (Poon and Day 1976). Given significant differences in nuclear division between Ascomycota and Basidiomycota, mechanisms of cytokinesis may also demonstrate a significant divergence between these phyla. For instance, in *S. cerevisiae* activation of MEN is triggered by the translocation of the SPB-associated Tem1 to the daughter cell in anaphase (Shirayama, Matsui et al. 1994, Bardin, Visintin et al. 2000, Pereira, Hofken et al. 2000), while in basidiomycetes SPBs are transitioned to the daughter cell prior to metaphase (Kozubowski, Yadav et al. 2013). It is puzzling how signaling based on Cdc14 release from the nucleolus would operate in basidiomycetes if the nucleolus disintegration initiates before the spindle assembles (Kozubowski, Yadav et al. 2013). Furthermore, a spindle position checkpoint could not operate in basidiomycetes if the Cdc14 were released from nucleolus and signaled to start cytokinesis before metaphase (Merlini and Piatti 2011). One possibility is that Cdc14-based signaling similar to MEN is not conserved in this phylum. On the other hand, both *C. neoformans* and *U. maydis* contain a homologue of the Cdc14 phosphatase and other typical MEN components (Table). Cdc14-like phosphatases are likely critical for the mitotic exit control in metazoans and may localize to the nucleolus (Bembenek and Yu 2001, Kaiser, Nachury et al. 2004). Therefore, the Cdc14 homologue may act in the mitotic exit network in basidiomycetes via mechanisms similar to that occurring in metazoans. Another non-exclusive possibility is that in basidiomycetes cytokinesis is triggered by mechanisms that are similar to the SIN in *S. pombe*. Hypothetically, in basidiomycetous yeasts the Cdc14/Clp1 homologue could get released from the nucleolus upon nucleolar disintegration prior to metaphase, but the actual signal that triggers cytokinesis would take effect in anaphase and be responsible for the sustained cytoplasmic localization.

Interestingly, in *U. maydis* deletion of genes encoding proteins homologous to the SIN components, Cdc14, Sid1, or Spg1 leads to the inhibition of the NE breakdown during mitosis (Straube, Weber et al. 2005, Sandrock, Bohmer et al. 2006). This suggests that in *U. maydis*, a signaling similar in architecture to SIN regulates NE breakdown. On the other hand, deletion of *ras3* in *U. maydis* did not result in a septation defect suggesting that this pathway is not essential for cytokinesis (Straube, Weber et al. 2005). Potentially this signaling could trigger an initial release of Clp1 homologue from the nucleolus, which could then potentiate further NE breakdown. Such a scenario may be also conserved in metazoans as metazoan Clp1 homologues are localizing to the nucleolus and are involved in cell division (Kaiser, Nachury et al. 2004). Sandrock et al. have proposed that Don3 has a dual role during cell division, regulating NE breakdown as a heterodimer with Dip1 and initiating the secondary septum as a homo-oligomer independent of Dip1 (Sandrock, Bohmer et al. 2006). This would suggest that in *U. maydis*, components homologous to the members of the SIN pathway play diverse roles in mitosis. It would be of interest to uncover the exact architecture of this signaling network and investigate its conservation in other basidiomycetous yeasts, including *C. neoformans*.

3. The Actomyosin Ring

The actomyosin ring (AMR) is composed of myosin II (heavy, light, and regulatory chains) and filamentous actin (F-actin) (Satterwhite and Pollard 1992). How the AMR components localize to the site of division and how the AMR assembles and finally constricts separating the cytoplasm of the dividing cells are key questions many studies have investigated utilizing the two model yeasts.

3.1 Myosin recruitment

Nonmuscle myosin II is the essential motor that drives actin-based cytokinesis (Sellers 2000). *S. cerevisiae* genome encodes one myosin II heavy chain, Myo1, and *S. pombe* encodes two, Myo2 and Myp2 (Mulvihill and Hyams 2003, Wloka and Bi 2012). While *MYO1* in *S. cerevisiae* is not essential, *MYO2* deletion in *S. pombe* results in inviable cells that do not form an AMR (Kitayama, Sugimoto et al. 1997, Bi, Maddox et al. 1998). Intriguingly, Myp2 in *S. pombe* is essential for cytokinesis only under nutrient-limiting or stress conditions (Bezanilla, Forsburg et al. 1997). The following are the essential myosin-related proteins for AMR assembly in *S. cerevisiae* and *S. pombe*: myosin II heavy chain, myosin II light chain, and IQGAP protein.

In *S. cerevisiae*, an initial recruitment of Myo1 to the mother-bud neck before cytokinesis depends on septins, filament-forming GTPases important for cytokinesis in animals and fungi (Section 6) (Juanes and Piatti 2016). During G1, the septin-Myo1 linker, Bni-5, is responsible for Myo1 localization at the bud neck (Fang, Luo et al. 2010). However, during cytokinesis, Myo1 is recruited to the bud neck by the myosin light chain (Mlc1) and IQGAP protein (Iqg1) via exocytosis-based mechanism involving formin Bni1, F-actin, and Myo2 in a septin-independent manner (Fang, Luo et al. 2010)(Boyne, Yosuf et al. 2000)(Feng, Okada et al. 2015).

Septins in *S. pombe* are not required for myosin II localization. Instead the anillin-like protein, Mid1, localizes to the cell middle and organizes cortical nodes consisting of IQGAP protein (Rng2), myosin II (Myo2), a formin (Cdc12), F-BAR protein (Cdc15), and myosin light and regulatory chains (Cdc4 and Rlc1) (Wu, Sirotkin et al. 2006, Willet, McDonald et al. 2015). Rng2 acts downstream of Mid1 and is essential for the recruitment of Myo2 (Laporte, Coffman et al. 2011, Padmanabhan, Bakka et al. 2011, Takaine, Numata et al. 2014). In the *mid1* deletion mutant, AMR assembly still occurs, but the ring positioning within the cell is affected (Bahler, Steever et al. 1998). *S. cerevisiae* anillin homologue, Bud4, is not acting in AMR assembly and instead participates in septin organization similar to the second anillin, Mid2, in *S. pombe* (Eluere, Varlet et al. 2012).

How myosin is recruited to the site of division has not been thoroughly studied in basidiomycetes. The *U. maydis* orthologue of the *S. pombe* myosin light chain Cdc4p localizes to the septin collar prior to mitosis and the reorganization of septins is necessary for the subsequent incorporation of Cdc4, along with the F-actin, into the AMR (Shannon and Li 2000, Bohmer, Ripp et al. 2009). In *C. neoformans*, Myo1 localizes to the bud neck and constricts similarly to the *S. cerevisiae* homologue (Aboobakar, Wang et al. 2011). *C. neoformans* genome encodes an anillin homologue, which may participate in septin

organization similar to Bud4 and Mid2; the *U. maydis* homologue candidate seems to be relatively less conserved (Table).

3.2 Assembly of AMR and the mechanisms of constriction

The AMR consists of antiparallel filaments of actin that undergo a continuous cycle of nucleation, polymerization, and depolymerization (Meitinger and Palani 2016). In *S. cerevisiae* and *S. pombe*, the actin-related components essential for AMR assembly are: formin, tropomyosin, and profilin. In *S. pombe*, potentially another component, the F-BAR protein Cdc15, plays an important role in AMR formation (Fankhauser, Raymond et al. 1995, Carnahan and Gould 2003), although the requirement of Cdc15 for the AMR assembly depends on the stage of mitosis (Wachtler, Huang et al. 2006) (see also section 4.2).

Formins are required for actin nucleation during polarized cell growth and cytokinesis (Evangelista, Zigmond et al. 2003). In *S. cerevisiae*, Bni1 is the essential formin, which promotes actin polymerization at sites of polarized growth and reassembles at the mother-bud neck during the onset to cytokinesis where it is crucial for the AMR assembly (Vallen, Caviston et al. 2000, Tolliday, VerPlank et al. 2002, Kovar 2006). *S. cerevisiae* encodes also another formin, Bnr1, that localizes at the bud neck prior to cytokinesis and is dispensable for AMR assembly and constriction (Moseley and Goode 2006). The *S. pombe* essential formin, Cdc12, plays a role in actin nucleation during AMR formation (Willet, McDonald et al. 2015). Additionally, tropomyosin and profilin stabilize formin nucleation and contribute to actin polymerization essential in AMR formation in model yeasts (Liu and Bretscher 1989, Drees, Brown et al. 1995, Sagot, Rodal et al. 2002).

The F-actin localization and dynamics in *C. neoformans* and *U. maydis* appear similar to *S. cerevisiae* (Kopecka, Gabriel et al. 2001, Banuett and Herskowitz 2002). While tropomyosins have not been thoroughly described in basidiomycetes, *C. neoformans* mutants lacking formin or tropomyosin homologues exhibit defects in cytokinesis (Chang, Lamichhane et al. 2012). *U. maydis* encodes two formin homologues: an essential formin Srf1, and a non-essential formin, Drf1. Drf1 acts as an effector of the Rho GTPase Cdc42 specifically during the AMR assembly during the secondary septation, and is needed for cell separation (Freitag, Lanver et al. 2011). Thus, it is likely that the AMR in basidiomycetes would have a similar architecture to the AMR in ascomycetes.

The search, capture, pull, and release (SCPR) mechanism of AMR formation in *S. pombe* describes the ring coalescing from approximately 60 cortical nodes (Wu, Sirotkin et al. 2006, Mishra and Oliferenko 2008, Vavylonis, Wu et al. 2008). Given that *C. neoformans* and *U. maydis* divide by budding, the mechanism of AMR assembly may be more similar to that described for *S. cerevisiae* (Meitinger and Palani 2016). Interestingly, cytokinesis in *U. maydis* involves the assembly of two AMRs, each coordinated by a distinct signaling (Bohmer, Bohmer et al. 2008, Bohmer, Ripp et al. 2009) (Sections 4 and 6). While the components responsible for the first AMR formation remain largely uncharacterized, germinal center kinase, Don3, and a Rho GTPase, Cdc42, participate in the assembly of the second AMR (Bohmer, Bohmer et al. 2008).

Two mechanisms have been shown to contribute to the constriction of the AMR in fungal cells (Balasubramanian, Srinivasan et al. 2012, Meitinger and Palani 2016). The first mechanism relies on the motor domain of the myosin, which similarly to the striated muscle contraction slides along the actin filaments. The second mechanism relies on the coordinated crosslinking and depolymerization of actin filaments (Lord, Laves et al. 2005, Fang, Luo et al. 2010, Mendes Pinto, Rubinstein et al. 2012, Wloka, Vallen et al. 2013, Juanes and Piatti 2016). This complex two-way mechanism appears to be conserved in metazoans (Ma, Kovacs et al. 2012) and likely occurs in basidiomycetes.

In model yeasts the AMR constriction is coincidental with the formation of the primary septum (Rincon and Paoletti 2016). Whether the AMR drives the septum formation directly, remains unclear. A study by Proctor et al. proposed that in *S. pombe* the essential contribution to cytokinesis of myosin and F-actin is restricted to only an initial part of the constriction process (Proctor, Minc et al. 2012). Thus, the major force in cytokinesis is likely attributed to the assembly of cell wall polymers in the growing septum; the AMR plays only a minor role. Given that all fungal cells have cell walls and form septa during cytokinesis it is likely that the mechanism of the constriction of the AMR and its relative contribution to cytokinesis are conserved in most fungi, including basidiomycetes.

4. Formation of the primary septum

The fungal septum is made of a cell wall-like material that ultimately forms a physical barrier separating the cytoplasm (Walther and Wendland 2003). The septum grows centripetally inward as the AMR closes. Optimal AMR constriction and primary septum formation are interdependent; AMR guides septum formation, while septum formation stabilizes AMR constriction (Vallen, Caviston et al. 2000, Schmidt, Bowers et al. 2002, VerPlank and Li 2005, Fang, Luo et al. 2010, Meitinger and Palani 2016). In *C. neoformans*, similar to *S. cerevisiae*, cytokinesis involves formation of a single primary septum followed by two secondary septa formed at each side of the primary septum (Kozubowski and Heitman 2010). In contrast, septation in *U. maydis* proceeds in a way that is distinct from *S. cerevisiae* (Weinzierl, Leveleki et al. 2002) (Figure 2). First, a primary septum forms on the mother side of the bud neck, which is then followed by the formation of a single secondary septum on the daughter side through transfer of vesicles from the daughter cell. Secondary septum delimits the fragmentation zone, which is an extracellular compartment filled with vesicles. Formation of the fragmentation zone initiates cell separation, which proceeds through hydrolytic degradation of the secondary septum (Weinzierl, Leveleki et al. 2002). Interestingly, while the first septation in *U. maydis* is coordinated with the cell cycle progression, the second septation is independent of the cell cycle and the two septation events are regulated by distinct signaling pathways (Bohmer, Bohmer et al. 2008) (Section 6).

4.1 Composition of the primary septum

Cell walls of most fungi contain chitin, a polymer of β -1,4 linked N-acetylglucosamine. While typically for yeast cells chitin constitutes 1–2% of the dry weight, it has not been detected in *S. pombe* (Sietsma and Wessels 1990). In both *U. maydis* and *C. neoformans* the

chitin content is significantly higher as compared to other yeasts (Ruiz-Herrera, Leon et al. 1996, Banks, Specht et al. 2005). Composition of the primary septum also varies among fungi. In *S. cerevisiae*, the primary septum consists of chitin, which is synthesized by chitin synthase Chs2. In contrast, septum formation in *S. pombe* involves the β -glucan synthases Bgs1/Cps1/Drc1, Bgs3, and Bgs4 (Ishiguro, Saitou et al. 1997, Nakano, Arai et al. 1997, Le Goff, Utzig et al. 1999, Cortes, Carnero et al. 2005) and the α -glucan synthase Ags1/Mok1 (Hochstenbach, Klis et al. 1998), which are regulated by the Rho GTPases Rho1 and Rho2 and the protein kinase C isoforms Pck1 and Pck2 (Arellano, Duran et al. 1996, Arellano, Duran et al. 1997, Nakano, Arai et al. 1997, Arellano, Valdivieso et al. 1999). Both, *C. neoformans* and *U. maydis* encode 8 chitin synthases, which reflects their morphologically complex life cycles (Gold and Kronstad 1994, Xoconostle-Cazares, Leon-Ramirez et al. 1996, Xoconostle-Cazares, Specht et al. 1997, Munro and Gow 2001, Garcera-Teruel, Xoconostle-Cazares et al. 2004, Banks, Specht et al. 2005, Weber, Assmann et al. 2006).

In *S. cerevisiae*, the major point of regulation during the formation of the primary septum is the targeted secretion and activation of the chitin synthase Chs2, dependent on the components of MEN (Chuang and Schekman 1996, VerPlank and Li 2005, Chin, Bennett et al. 2012, Oh, Chang et al. 2012, Rogg, Fortwendel et al. 2012, Jakobsen, Cheng et al. 2013, Meitinger and Palani 2016). Chs2 is delivered on vesicles via class V unconventional myosin Myo2- and actin-dependent transport. *S. pombe* encodes two class V myosins and one of them, Myo52 is associated with the septum and contributes to the delivery of septum components similar to the role of Myo2 in *S. cerevisiae* (Win, Gachet et al. 2001).

The only myosin type V in *U. maydis*, Myo5 accumulates at the cleavage site, and is most likely involved in septum formation or cell separation (Weber, Gruber et al. 2003). Similar to *S. pombe myo52* mutants, *myo5* cells are viable but grow slowly and frequently fail to separate, forming large cell aggregates divided by septa (Weber, Gruber et al. 2003). The identity of the putative cargo delivered by Myo5 in *U. maydis* remains unknown (Weber, Gruber et al. 2003). While all of the 8 chitin synthases encoded by *U. maydis* are expressed in yeast cells and localize to the septum, only mutants in the class IV chitin synthase genes *CHS5* and *CHS7* exhibit morphological defects in yeast cells. The *chs7* cells display a more significant cell separation defect, suggesting that these enzymes share redundant functions in septum formation (Weber, Assmann et al. 2006). Similar to *U. maydis*, none of the eight encoded chitin synthases and three putative chitin synthase regulators in *C. neoformans* are essential for viability (Banks, Specht et al. 2005). Moreover, a class IV chitin synthase, homologue of *S. cerevisiae* Chs3 and its putative regulator Csr2 were required for completion of cell separation (Banks, Specht et al. 2005). Thus, in both basidiomycetous yeasts class IV chitin synthases appear important for the formation of the primary septum.

Chitosan, the deacetylated more soluble derivative of chitin, is produced enzymatically by chitin deacetylases. Unlike *S. cerevisiae*, in which chitosan appears only during sporulation (Briza, Ellinger et al. 1988), *C. neoformans* contains significant amounts of chitosan in vegetative cells (Banks, Specht et al. 2005), and its loss results in slower growth and a defect in daughter cell separation suggesting an important role during cytokinesis (Baker, Specht et al. 2007). No other basidiomycetes have been reported to contain chitosan and it would be of

interest to probe if this is limited to *C. neoformans*. One study indicated an absence of chitosan in *U. maydis* (Ruiz-Herrera, Leon et al. 1996). However, the *U. maydis* genome contains homologues of all three *C. neoformans* chitin deacetylases (UM02689, UM02019, UM00638) that may potentially contribute to chitosan production. Therefore, it is possible that similar to *C. neoformans* chitosan may be necessary for the efficient cell separation in other basidiomycetous yeasts.

4.2 Coordination of septum formation with the AMR constriction

In both model yeasts, a complex of F-BAR proteins, C2 domain proteins, and associated factors localizes to the site of septum formation and couples septum formation to the membrane ingression and the AMR constriction (Juanes and Piatti 2016, Rincon and Paoletti 2016). In *S. pombe*, the F-BAR proteins Cdc15, Imp2, and Rga7, with the C2 domain protein Fic1, a paxilin homolog Pxl1, and Cyk3 form a complex to coordinate AMR constriction with the septum formation, whereas in *S. cerevisiae* a single F-BAR protein Hof1 interacts with the C2 domain protein Inn1 and Cyk3 to play an analogous role (Sanchez-Diaz, Marchesi et al. 2008, Jendretzki, Ciklic et al. 2009, Nishihama, Schreiter et al. 2009, Roberts-Galbraith, Chen et al. 2009, Pollard, Onishi et al. 2012). These proteins are subject to complex regulation and potentially cooperate with yet more unidentified factors to achieve optimal septum formation (Juanes and Piatti 2016, Rincon and Paoletti 2016). *S. cerevisiae* Hof1 is not essential for viability and the AMR constriction (Vallen, Caviston et al. 2000), whereas *S. pombe* Cdc15 plays a key role early in the establishment of the AMR (Fankhauser, Reymond et al. 1995, Carnahan and Gould 2003).

Putative homologues of the F-BAR protein and the C2 domain protein are encoded by the *C. neoformans* and *U. maydis* genomes, while there is no significant homologue of Cyk3 (Table). The *U. maydis* F-BAR domain protein Cdc15 was not required for the recruitment of the essential light chain of the myosin II, Cdc4, and the F-actin to the sites of septation but it was necessary for the subsequent constriction of Cdc4 along with F-actin, indicating that Cdc15 is not essential for cell viability and organizing actin-based ring at cytokinesis but is required for the constriction of the AMR (Bohmer, Ripp et al. 2009). *U. maydis*, *cdc15* mutants formed long chains of cells that were connected by unusually broad septa, which is analogous to the *hof1* phenotype in *S. cerevisiae* (Bohmer, Ripp et al. 2009).

C. neoformans C2 domain protein Cts1 (for calcineurin mutant temperature sensitivity suppressor 1) is homologous to the *S. pombe* Fic1 and *S. cerevisiae* Inn1 (Fox, Cox et al. 2003, Roberts-Galbraith, Chen et al. 2009, Aboobakar, Wang et al. 2011). Cts1 localizes to the site of cytokinesis and the deletion of the *CTS1* results in cell separation defect (Fox, Cox et al. 2003, Aboobakar, Wang et al. 2011). Specifically the *cts1* mutant cells did not form typical primary septa and instead accumulated an abnormally thick septal material, similar to *S. cerevisiae inn1* mutant (Fox, Cox et al. 2003, Nishihama, Schreiter et al. 2009). Interestingly, the constriction of Cts1 appeared to follow rather than coincide with the constriction of the AMR (Aboobakar, Wang et al. 2011), in contrast to Inn1 (Nishihama, Schreiter et al. 2009). Whether Inn1 depends on AMR for localization remains controversial and it would be of interest to test it for the Cts1 in *C. neoformans* (Sanchez-Diaz, Marchesi et al. 2008, Nishihama, Schreiter et al. 2009). In model yeasts, the C2 domain proteins bind

to the SH3 domain of the F-BAR proteins (Nishihama, Schreiter et al. 2009, Rincon and Paoletti 2016). Cts1 is rich in prolines, which is consistent with a possible interaction with SH3 domains (Nguyen, Turck et al. 1998). Whether Inn1 directly binds to membranes through the C2 domain remains debatable as no phospholipid binding of the purified Inn1 was detected (Sanchez-Diaz, Marchesi et al. 2008, Nishihama, Schreiter et al. 2009). In contrast, Cts1 binds phosphatidylinositol-5-phosphate (PI(5)P) and PI(4)P in a C2-dependent manner (Fox, Cox et al. 2003). Thus, while it is possible that Cts1 carries out functions analogous to Inn1 and Fic1, the exact way it contributes to septum integrity may be unique. Interestingly, Cts1 may be a substrate and an effector of the calcium-dependent phosphatase calcineurin during high-temperature stress (Roy and Cyert 2009, Aboobakar, Wang et al. 2011). In *S. pombe*, calcineurin has been shown to play an important role in septation (Yoshida, Toda et al. 1994). Thus, further studies in *C. neoformans* may help to elucidate the connection between septum formation and calcineurin signaling. In addition, Cts1 may play a role in vacuolar membrane trafficking, as GFP-Cts1 co-localizes with late endosomes and the *cts1* strain showed altered dynamics of endocytosis (Aboobakar, Wang et al. 2011) consistent with previous reports on roles of C2 domain proteins in membrane trafficking (Sudhof and Rizo 1996, Burns, Sasaki et al. 1998). Interestingly, the *U. maydis* Cdc42-specific guanine nucleotide exchange factor Don1 localizes to fast-moving endosomal vesicles that accumulate at the site of septation (Schink and Bolker 2009, Gohre, Vollmeister et al. 2012). Perhaps C2 domain proteins contribute to cytokinesis by both directly participating in septum dynamics and coordinating endosomal trafficking of proteins involved in cell separation.

5. Formation of the secondary septum and final cell separation

In model yeasts, secondary septa constitute a new cell wall that is deposited on both sides of the primary septum. Secondary septum formation involves extensive delivery of vesicles to the bud neck and the activity of glucan synthases and also chitin synthase in case of *S. cerevisiae* (Cabib, Roh et al. 2001). In the absence of primary septum synthesis or AMR constriction, a synthesis of a “remedial secondary septum” is sufficient to separate mother and daughter cells (Cabib and Schmidt 2003). Transmission Electron Microscopy studies show analogous septal structures in *C. neoformans*, suggesting mechanisms responsible for the synthesis of both septa are similar to that in *S. cerevisiae* (Kozubowski and Heitman 2010). In contrast, *U. maydis* forms a single secondary septum, whose assembly may proceed similar to the assembly of the primary septum (Weinzierl, Leveleki et al. 2002) (Figure 2B).

The final daughter cell detachment in fungi is accomplished by the concerted action of hydrolytic enzymes that degrade the thin layer of the primary septum (Adams 2004) and is controlled by a highly conserved morphogenesis-related pathway (MOR) also known as the RAM (regulation of Ace2 and morphogenesis) pathway (Maerz and Seiler 2010). Similar to MEN, the RAM pathway consists of a kinase cascade, including upstream germinal center kinase (GCK), which controls an Dbf2-related (NDR) kinase associated with the “Mob” subunit. While the core architecture of RAM is highly conserved, the downstream effectors vary (Maerz and Seiler 2010). In *S. cerevisiae* the NDR kinase, Cbk1, stimulates the Ace2 transcription factor to facilitate cell separation (Weiss 2012). In addition to the

transcriptional control, Cbk1 also directly negatively regulates Ssd1, a protein, which inhibits translation of hydrolytic enzymes by binding mRNA's (Jansen, Wanless et al. 2009). Similar to *S. cerevisiae*, both *U. maydis* and *C. neoformans*, encode homologues of the NDR kinase and the associated Mob protein and the deletion of these genes leads to cell separation defects (Durrenberger and Kronstad 1999, Walton, Heitman et al. 2006, Sartorel and Perez-Martin 2012). Interestingly, inactivation of the RAM pathway in these basidiomycetous yeasts leads to a hyperpolarized phenotype instead of inhibition of the polarized growth as observed in *S. cerevisiae*, suggesting rewiring of the morphogenesis related effectors of RAM during fungal evolution (Sartorel and Perez-Martin 2012).

U. maydis encodes 4 enzymes with the chitinase activity. Two of the enzymes Cts1 and Cts2 were required for cell separation (Langner, Ozturk et al. 2015). Interestingly, the growth rates of the *cts1/cts2* double mutant and the wild type were comparable suggesting no inhibition of the cell cycle due to inhibited cell separation (Langner, Ozturk et al. 2015). Cts1 is distributed asymmetrically within the fragmentation zone at the time when only the mother-derived primary septum is present, which suggests that it is delivered specifically from the daughter cell (Langner, Ozturk et al. 2015). In *S. cerevisiae*, the Ace2 transcription factor accumulates specifically in the daughter nucleus and promotes hydrolysis of the septum from the daughter side (Weiss 2012). Interestingly no clear homologues of Ace2 are present in *U. maydis* or *C. neoformans* genomes. This suggests alternative mechanisms for positioning the activity of the hydrolytic enzymes specifically on the daughter side. Surprisingly, none of the endochitinases encoded by the *C. neoformans* genome are necessary for vegetative growth consistent with an absence of the bud scar in this yeast (Banks, Specht et al. 2005, Baker, Specht et al. 2009). As *C. neoformans* may not have an enzyme that specifically hydrolyzes chitosan, an alternative mechanism for daughter cell separation that is based on the increased flexibility and solubility of the chitosan has been proposed (Baker, Specht et al. 2009).

6. The septin complex and cytokinesis

Septins are filament forming GTP-binding proteins that assemble into higher order structures at the cell cortex and have been implicated in cytokinesis in all eukaryotes, except plants (Mostowy and Cossart 2012, Fung, Dai et al. 2014, Bridges and Gladfelter 2015). In model yeasts, septin higher order structures are necessary for septin function and often rearrange during the cell cycle (McMurray, Bertin et al. 2011). In *S. cerevisiae*, septins form a patch and then a ring at the incipient bud site, which subsequently rearranges into a collar at the mother-bud neck (Gladfelter, Pringle et al. 2001). During mitosis, the septin collar undergoes a structural change to form two separate rings each positioned on one side of the mother-bud neck (Gladfelter, Pringle et al. 2001).

6.1 Assembly and dynamics of the septin complex

Despite recent advances, it remains unclear how septin filaments are formed and subsequently organized into higher order structures (Zhang, Kong et al. 1999, Bridges, Zhang et al. 2014, Bridges and Gladfelter 2015). In *S. cerevisiae*, the assembly of higher order septin structures is coordinated by Rho GTPases and their effector proteins and may be

influenced by membrane properties and local exocytosis (Gladfelter, Bose et al. 2002, Caviston, Longtine et al. 2003, Iwase, Luo et al. 2006, Okada, Leda et al. 2013, Sadian, Gatsogiannis et al. 2013). Once assembled, septin-based structures undergo rearrangements during the cell cycle and the mechanisms responsible involve phosphorylation of septins and associated signaling proteins (Vrabioiu and Mitchison 2006, Ong, Wloka et al. 2014).

Both *U. maydis* and *C. neoformans* encode homologues of 4 vegetative septins initially described in *S. cerevisiae* (Cdc3, Cdc10, Cdc11, and Cdc12) (Boyce, Chang et al. 2005, Kozubowski and Heitman 2010, Alvarez-Tabares and Perez-Martin 2010, Bridges and Gladfelter 2014), and lack homologues of two sporulation specific septins and the fifth vegetative septin Shs1 whose supporting role in cytokinesis has been demonstrated in *S. cerevisiae* (Pan, Malmberg et al. 2007). The mother-bud neck localization appears conserved in basidiomycetous yeasts; in *C. neoformans*, septins undergo changes that appear very similar to those described in *S. cerevisiae* (Haarer and Pringle 1987, Kozubowski and Heitman 2010) (Figure 2A). Unlike in *C. neoformans*, the septin collar does not split into two rings during cytokinesis in *U. maydis* (Bohmer, Ripp et al. 2009, Alvarez-Tabares and Perez-Martin 2010)(Figure 2). Instead, formation of both septa in *U. maydis* proceeds with the following septin dynamics. First, the septin collar disassembles and the AMR components assemble. Next, septins re-assemble as a single ring on one side of the future septum and remain there after the AMR constricts (Figure 2B)(Bohmer, Ripp et al. 2009).

What signaling pathways lead to the assembly of septin higher order structures in basidiomycetous yeasts remains largely uncharacterized. In an elegant study, with the use of the analogue-sensitive variant of the germinal center kinase Don3, Bohmer et al demonstrated that the formation of the secondary septum in *U. maydis* is uncoupled from the mitotic exit and depends on Don3 and Cdc42 (Bohmer, Bohmer et al. 2008). In subsequent studies, the authors showed that Don3 is crucial for the disassembly of the septin collar, which is necessary for the assembly of the AMR (Bohmer, Ripp et al. 2009). On the other hand, the reassembly of septins into a ring is dependent on the AMR formation, coordinated by the Don1-Cdc42-Drf1 signaling (Freitag, Lanver et al. 2011)(Figure 2B). Whether Cdc42 is directly involved in the assembly of septins into a collar during the secondary septum formation remains unclear (Bohmer, Bohmer et al. 2008, Bohmer, Ripp et al. 2009, Freitag, Lanver et al. 2011).

U. maydis cells deleted for the gene encoding the PAK family kinase Cla4, a putative effector of Cdc42 and Rac1, grew as unusually shaped and branched hyphae consisting of uninuclear compartments separated by only single septa, a phenotype reminiscent of the morphological defects associated with single septin deletions (Leveleki, Mahlert et al. 2004). In *cla4* cells, actin failed to polarize to the sites of septation, suggesting that Cla4 is necessary for the AMR assembly (Leveleki, Mahlert et al. 2004). A similar phenotype also resulted from the deletion of the gene encoding GTPase Rac1 in *U. maydis* (Leveleki, Mahlert et al. 2004, Mahlert, Leveleki et al. 2006). Thus, it is plausible that in *U. maydis*, Rac1 and its putative effector Cla4 are responsible for the assembly of the septin higher order structures to support primary septum formation. The resulting phenotype of the mutants affected in this pathway would constitute formation of only remedial single septa that are formed in the absence of septins and the AMR. Such a possibility would somewhat

contradict the findings that certain double septin mutants are inviable (Alvarez-Tabares and Perez-Martin 2010)(section 6.2). However, it is possible that in the absence of the putative Rac1/Cla4 signaling, septin complexes still assemble at the division site albeit with compromised architecture. In addition, the essential role of some of the septins may not be associated with cytokinesis in *U. maydis* (Alvarez-Tabares and Perez-Martin 2010).

In *C. neoformans*, a signaling network consisting of the upstream GTPase Ras1 and four downstream GTPases forming two paralogous pairs, Rac1 with Rac2 and Cdc42 with Cdc420, act to regulate cell polarity and cytokinesis during stress (Ballou, Kozubowski et al. 2013). In this pathway, Cdc42/Cdc420 are responsible for the assembly of the septin complex at the bud neck (Ballou, Nichols et al. 2009). Over-expression of Cdc42 restored septin protein localization to the *ras1* mutant and also suppressed *ras1* mutant defects in budding at 37°C (Ballou, Kozubowski et al. 2013). As *cdc42 cdc420* double mutant is viable at non-stress conditions, this suggests that higher order septin assemblies at the bud neck are dispensable for cell proliferation of *C. neoformans*, unless cells are exposed to higher temperature or other stresses (Section 6.2). Interestingly, the Cdc42-dependent septin organization is not mediated by the presumed effector of Cdc42, the Cla4 homologue in *C. neoformans*, despite the deletion mutant having a clear cytokinesis defect at high temperature (Nichols, Perfect et al. 2007, Ballou, Nichols et al. 2009).

6.2 Are septin higher order structures at the mother bud neck essential for cytokinesis and viability in basidiomycetes?

Two primary roles of septins are to act as a scaffold and to compartmentalize the cell cortex by forming diffusion barrier against membrane associated proteins (Caudron and Barral 2009, Bridges and Gladfelter 2015). In *S. cerevisiae*, septins support nearly 60 proteins at the mother-bud neck, including Myo1 and several other cytokinesis proteins (Wloka and Bi 2012, Bridges and Gladfelter 2015, Juanes and Piatti 2016). It remains controversial whether the diffusion barrier formed by the septin ring is necessary for cytokinesis in *S. cerevisiae* (Dobbelaere and Barral 2004, Wloka, Nishihama et al. 2011). While in numerous organisms septins are essential (presumably because of their essential contribution to cytokinesis), in some cell types septins are dispensable (Menon and Gaestel 2015). For instance, septins are required for cytokinesis and viability in *S. cerevisiae* but they are dispensable in *S. pombe*, potentially reflecting the difference between the two modes of cell division of these yeasts (Bi, Maddox et al. 1998, Iwase, Luo et al. 2007, Bridges and Gladfelter 2015, Finnigan, Booth et al. 2015).

Neither of the four septins is essential for viability in *U. maydis* and *C. neoformans* (Boyce, Chang et al. 2005, Kozubowski and Heitman 2010, Alvarez-Tabares and Perez-Martin 2010). This is in contrast to *S. cerevisiae* where elimination of either septin Cdc3 or Cdc12 is lethal, presumably due to a significant defect in the formation of the septin complex. *S. cerevisiae* septin Cdc10 is not essential and in *cdc10* cells the septin complex still forms, although efficient ring splitting during cytokinesis is compromised (Wloka, Nishihama et al. 2011). Strikingly, elimination of both Cdc3 and Cdc12 homologues in *U. maydis* is not lethal at temperatures up to 28°C (Alvarez-Tabares and Perez-Martin 2010), potentially because Cdc10 and Cdc11 assemble in this mutant and provide the essential function (Alvarez-

Tabares and Perez-Martin 2010). Somewhat inconsistent with these results is that neither of the septins are detected in any of the single septin mutants at the bud neck at 28°C, a temperature at which all septin single mutants are still viable (Alvarez-Tabares and Perez-Martin 2010). On the other hand, the *cdc10*, *cdc3* and *cdc10*, *cdc11* double mutants are inviable even at low temperature, suggesting that neither Cdc11 with Cdc12 or Cdc3 with Cdc12 can assemble at the bud neck and/or provide an essential function at 22°C (Alvarez-Tabares and Perez-Martin 2010). Potentially the essential role of some of the septins in *U. maydis* is not related to cytokinesis as septins were found at other locations in addition to the bud neck in this species (Alvarez-Tabares and Perez-Martin 2010). At 34°C, the *cdc3* and *cdc12* single septin mutants are inviable, while the growth of *cdc10* and *cdc11* single mutants are significantly compromised (Alvarez-Tabares and Perez-Martin 2010). Hypothetically, two non-exclusive possibilities could account for the temperature dependence of the septin deletion phenotypes in *U. maydis*. First, temperature may influence the degree to which the septin complex structure is compromised. Alternatively, at higher temperatures a lack of the process that the septins normally provide becomes detrimental to growth. Investigating specific defects in cell division, including the AMR assembly and dynamics, and chitin synthase localization in septin mutants at various temperatures may help to distinguish between these possibilities.

It appears that in *C. neoformans* at 25°C, septin complex at the bud neck is not essential for viability, as the *cdc3 cdc12* double mutant is viable at low temperatures, despite the absence of the remaining septins at the site of cytokinesis (Kozubowski and Heitman 2010). Therefore it is likely that in *C. neoformans* septin-dependent processes are not essential for viability at low temperatures and only become essential at higher temperatures or during other stresses. Examining specific cytokinesis events in septin mutants at low temperature will help to test this hypothesis. Thus, it appears that both *U. maydis* and *C. neoformans* evolved pathways that are redundant to septin function for the completion of cytokinesis and cell separation. Such a redundancy may be more common to fungal pathogens rather than specific to basidiomycetous yeast (Walker, Lenardon et al. 2013, Bridges and Gladfelter 2014).

7. Summary

While our knowledge is still very incomplete, several interesting facts about cytokinesis in basidiomycetes begin to emerge as indicated in Highlights. *C. neoformans* and *U. maydis* display common features of cytokinesis that may be unique in basidiomycetes, including, non-essential role of septins for instance. On the other hand, some aspects are distinct between the two species and likely specific to their respective classes, for instance the architecture of septation. Furthermore, both *C. neoformans* and *U. maydis* are pathogens and potentially their mechanisms of cytokinesis may have evolved to accommodate to host conditions. The continual efforts in cell biological approaches involving basidiomycetes, including also non-pathogenic species, should aid in our understanding of the plasticity of this process in fungal kingdom and help to elucidate mechanisms of cytokinesis, including the coordination of this process with other parts of cell division and the responses to environmental stimuli.

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Abbreviations

AMR	actomyosin ring
NE	nuclear envelope
SPB	spindle pole body
MEN	mitotic exit network
SIN	septation initiation network

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Highlights

- Basidiomycetous yeasts undergo open mitosis.
- Entry into cytokinesis may be controlled by pathways similar to metazoans.
- *C. neoformans* forms primary septum and two secondary septa, similar to *S. cerevisiae*.
- *U. maydis* forms primary septum and a single secondary septum.
- Both yeasts have evolved alternative pathways operating in the absence of septins.

Figure 1. Key events during mitosis in *C. neoformans* and *U. maydis*.

In both yeasts, non-dividing cells (G2) contain a network of cytoplasmic microtubules (MT). In prophase cytoplasmic MT capture an outer plaque of the spindle pole bodies (SPBs) and pool the entire chromatin to the daughter cell. In metaphase, chromatin compacts and arranges around the spindle; at that time cytoplasmic MT disappear. In *C. neoformans* the nuclear envelope (NE) moves along with the chromatin to the daughter cell and partially breaks open. In *U. maydis* the NE stays in the mother cell and also disintegrates. Localization of the Nup107, which is the essential component of the Nuclear Pore Complexes (NPC), suggests that the NPCs disassemble from the NE during metaphase. In *U. maydis*, but not in *C. neoformans*, Nup107 is recruited subsequently to the chromatin. In late telophase, NE is rebuilt, NPCs re-assemble, and cytoplasmic microtubule network reappears (not shown).

Figure 2. Septin and AMR dynamics during the cell cycle

(A) *U. maydis* and *C. neoformans* septins are initially recruited to the incipient bud site and form a patch (not shown), which rearranges into a ring (G1). Typically in *U. maydis*, the S phase is completed before bud initiation, whereas budding in *C. neoformans* starts soon after beginning of the S phase and may be delayed until G2 in nutrient limiting conditions (Snetselaar 1997, Ohkusu, Hata et al. 2001). During bud growth (G2), in both yeasts, the septins form an hourglass-shaped collar at the mother-bud neck. During cytokinesis, in *C. neoformans* the septin collar re-arranges into two separate rings on both sides of the bud neck and the AMR assembles between the double septin ring. In contrast, in *U. maydis* the septin collar does not re-arrange into a double ring. Instead, septins undergo a structural change (as outlined in (B)), that is repeated twice for each of the two septa that are formed in *U. maydis*. (B) Schematic representing the neck region in *U. maydis* and the dynamics of septins and AMR during formation of the two consecutive septa. First, septin collar on the mother side of the bud neck disassembles, which is necessary for the assembly of the AMR. Next, septins reassemble into a ring on the mother side of the AMR. By the time the primary septum is formed, a second septin collar forms on the daughter side of the bud neck and the analogous septin re-arrangement takes place to support the formation of the secondary septum. Proteins identified to participate in each step are indicated.

C. neoformans var. *grubii* H99 (C.n.), *U. maydis* 521 (U.m.), and *S. pombe* 972h (S.p.) genomes were probed for similarity against *S. cerevisiae* S288c (S.c.) using FungiDB (<http://fungidb.org/fungidb/>). Recorded E-values were products of protein BLAST results from FungiDB. Function and protein sequences were retrieved from SGD (<http://www.yeastgenome.org>).

Table

S. c	S. p	C. n (CNAG #)	E-value	U. m. (UMAG #)	E-value	Function in <i>S. cerevisiae</i>	References
Tem1	Spg1	05513	3.00E-83	11050	1.00E-78	GTP-binding protein	U: (Straube, Weber et al. 2005)
Cdc15	Cdc7	06845	5.00E-58	00721	2.00E-59	protein kinase of MEN	
Mob1	Mob1	05541	9.00E-58	04352	2.00E-64	component of MEN	U: (Sartorel and Perez-Martin 2012)
Cdc14	Clp1/Flp1	00498	1.00E-83	06187	7.00E-90	protein phosphatase	
Sps1	Sid1	03290	6.00E-83	05543	9.00E-80	protein serine/threonine kinase	U: (Sandrock, Bohmer et al. 2006)
Dbf2	Sid2	02194	2.00E-157	03446	1.00E-151	protein serine/threonine kinase	
Myo1	Myo2 Myp2	01536	0.00E+00	03286	0.00E+00	type II myosin heavy chain	C: (Aboobakar, Wang et al. 2011)
Mlc1	Cdc4	00808	1.00E-29	11848	3.00E-36	myosin light chain	U: (Bohmer, Ripp et al. 2009)
Act1	Act1	00483	0.00E+00	11232	0.00E+00	actin	
Iqg1	Rng2	03763	2.00E-24	10730	3.00E-30	IQGAP-related protein	
Bni1	Cdc12	04815	7.00E-22	12254	4.00E-32	formin	C: (Chang, Lamichhane et al. 2012) U: (Freitag, Lanver et al. 2011)
Bnr1	Fus1	-	-	01141	1.00E-09	formin	U: (Freitag, Lanver et al. 2011)
Tpm1	Cdc8	05701	3.00E-18	11985	2.00E-21	tropomyosin	C: (Chang, Lamichhane et al. 2012)
Tpm2	Cdc8	05701	2.00E-16	11985	6.00E-24	tropomyosin	
Pfy1	Cdc3	00584	1.00E-24	10832	4.00E-32	profilin	
Cdc42	Cdc42	05348	1.00E-117	00295	2.00E-122	small rho-like GTPase	C: (Ballou, Nichols et al. 2009) U: (Bohmer, Bohmer et al. 2008)
Bud4	Mid2	06902	2.00E-08	UMAG12265	1.00E-56*	anillin-like protein	
Hof1	Cdc15, Imp2	06740	6.00E-12	00168	8.00E-09	F-bar protein	
Bni5	-	01051	3.40E+00	-	-	septin-Myo1 linker	
Chs1	Chs1	07499	0.00E+00	10117	0.00E+00	chitin synthase	C: (Banks, Specht et al. 2005) U: (Weber, Assmann et al. 2006)
Chs2	Chs2	03326	0.00E+00	04290	0.00E+00	chitin synthase	
Chs3	-	05581	0.00E+00	10277	0.00E+00	chitin synthase	
Chs4	Cfh1	07636	9.00E-83	10641	1.00E-69	activator of Chs3p	

S. c	S. p	C. n (CNAG #)	E-value	U. m. (UMAG #)	E-value	Function in <i>S. cerevisiae</i>	References
Fks1 (Gsc1)	Bgs4	06508	0.00E+00	01639	0.00E+00	subunit of 1,3-beta-D-glucan synthase	
Fks2 (Gsc2)	Bgs4	06508	0.00E+00	01639	0.00E+00	subunit of 1,3-beta-D-glucan synthase	
Rho1	Rho5	03315	3.00E-102	05734	8.00E-107	GTP-binding protein	
Myo2	Myo52	06971	0.00E+00	04555	0.00E+00	type V myosin heavy chain	U: (Weber, Gruber et al. 2003)
Inn1	Fic1	03422	3.00E-04	06398	8.00E-47*	C2 domain protein	C: (Aboobakar, Wang et al. 2011)
Cyk3	Cyk3	-	-	-	-	SH3 domain protein	
Cbk1	Orb6	03567	0.00E+00	04956	0.00E+00	protein serine/threonine kinase of RAM	U: (Sartorel and Perez-Martin 2012) C: (Walton, Heitman et al. 2006)
Ssd1	Sls5	03345	1.00E-129	01220	1.00E-158	translational repressor	
Mob2	Mob2	05794	4.00E-66	12135	1.00E-62	activator of Cbk1p kinase in RAM	U: (Sartorel and Perez-Martin 2012)
Kic1	Nak1	00405	5.00E-83	11396	2.00E-96	protein kinase of the PAK/Ste20 family	C: (Walton, Heitman et al. 2006)
Tao3	Mor2	03622	2.00E-147	10098	1.00E-154	RAM component	
Sog2	Sog2	03918	7.00E-14	02656	1.00E-15	RAM component	
Cdc3	Spn1	05925	3.00E-146	10503	5.00E-150	septin	C: (Kozubowski and Heitman 2010)
Cdc10	Spn2	01373	4.00E-135	10644	4.00E-122	septin	U: (Alvarez-Tabares and Perez-Martin 2010)
Cdc11	Spn3	02196	5.00E-112	03449	2.00E-104	septin	
Cdc12	Spn6	01740	3.00E-141	03599	4.00E-152	septin	
Exo84	Exo84	04339	3.00E-24	04147	9.00E-20	exocyst complex component	
Eng1 (Dse4)	Eng2	-	-	-	-	daughter cell specific secreted protein	

* The E-value based on similarity to the *C. neoformans* homologue.

• References refer to either *C. neoformans* (marked as C:) or *U. maydis* (U:)