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THE MACHINERY FOR CELL POLARITY, CELL MORPHOGENESIS, AND THE CYTOSKELETON IN THE BASIDIOMYCETE FUNGUS *Ustilago maydis* A SURVEY OF THE GENOME SEQUENCE[#]

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Abstract

Ustilago maydis, a Basidiomycete fungus that infects maize, exhibits two basic morphologies, a yeast-like and a filamentous form. The yeast-like cell is elongated, divides by budding, and the bud grows by tip extension. The filamentous form divides at the apical cell and grows by tip extension. The repertoire of morphologies is increased during interaction with its host, suggesting that plant signals play an important role in generation of additional morphologies. We have used *S. cerevisiae* and *S. pombe* genes known to play a role in cell polarity and morphogenesis, and in the cytoskeleton as probes to survey the *U. maydis* genome. We have found that most of the yeast machinery is conserved in *U. maydis*, albeit the degree of similarity varies from strong to weak. The *U. maydis* genome contains the machinery for recognition and interpretation of the budding yeast axial and bipolar landmarks; however, genes coding for some of the landmark proteins are absent. Genes coding for cell polarity establishment, exocytosis, actin and microtubule organization, microtubule plus-end associated proteins, kinesins, and myosins are also present. Genes not present in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* include a homologue of mammalian Rac, a hybrid myosin-chitin synthase, and several kinesins that exhibit more similarity to their mammalian counterparts. We also used the *U. maydis* genes identified in this analysis to search other fungal and other eukaryotic genomes to identify the closest homologues. In most cases, not surprisingly, the closest homologue is among filamentous fungi, not the yeasts, and in some cases it is among mammals.

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[#]In memoriam Ira Herskowitz

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Keywords

polarized growth; cytoskeleton; polarisome; fungal morphogenesis

I. OVERVIEW OF CELL POLARITY AND CELL ORPHOGENESIS

Cell polarity is a fundamental biological process by which asymmetry of cell components (cytoskeleton, cell organelles, and membrane domains) is generated. Polarization of cell components occurs in diverse cells in response to endogenous programming and to external cues. This cellular asymmetry is important in determining cell morphogenesis and the plane of cell division in a variety of eukaryotic cells, and is crucial for development of metazoans. Studies in budding yeast and fission yeast have been instrumental in identification of the machinery involved in cell polarity and morphogenesis. These studies indicate that recognition of a spatial landmark triggers assembly of protein complexes that initiate assembly and polarization of the actin cytoskeleton, which directs vesicle delivery to sites of growth.

Fungal filaments (hyphae) exhibit continuous polarized growth at their tips. New axes of polarized growth (branches) are established along the main axis of polarized growth. Current work in filamentous fungi aims to understand the molecular mechanisms that determine: (a) the axis of polarized growth, (b) new axes of cell polarity, and (c) the site of cytokinesis and septum formation. The availability of the genome sequences of fungi from across the different subdivisions of the kingdom, together with improved tools for generation of knock out strains, regulatable gene expression, and sophisticated imaging techniques promise to reveal new insights about polarized growth and cell morphogenesis in filamentous fungi, and will likely lead in the future to the generation of protein interaction maps for this machinery as has been done in *Saccharomyces cerevisiae* (Drees et al., 2001).

Here we explore the genome sequence of *Ustilago maydis* in search of homologues of genes known to play crucial roles in cell morphogenesis and cell polarity in the yeasts. We first provide a synopsis of the morphologies that characterize *U. maydis* growth in culture and in the plant. We follow with a brief summary of key players of polarized growth in *S. cerevisiae*, and indicate which components of the machinery are conserved in *U. maydis*, and where known, describe their role in polarized growth. We conclude from our analysis that most of the yeast machinery is conserved in *U. maydis*, as it is in other filamentous fungi (Harris, 2006; Philippsen et al., 2005), and in many instances it is used in novel ways.

II. OVERVIEW OF THE MORPHOLOGICAL TRANSITIONS IN THE LIFE CYCLE OF *U. maydis*

Ustilago maydis is a Basidiomycete fungus that infects maize (*Zea mays* L) and teosinte (*Zea mays* spp. *mexicana* or spp. *parviglumis*). The fungus exhibits two basic morphologies and is capable of switching from one form to the other (the dimorphic switch) (Fig. 1). This switch is crucial to pathogenicity. The yeast-like form is unicellular, haploid, divides by budding, and is nonpathogenic; the filamentous form is dikaryotic, grows by tip extension, and is pathogenic (reviewed in Banuett, 1995;2002;Christensen, 1963;Holliday, 1974;Klosterman et al., 2007). The dimorphic switch and other aspects of the life cycle are controlled by two unlinked mating type loci, *a* and *b*. The *a* locus codes for components of a signal transduction pathway (pheromone precursor and receptor genes) and the *b* locus codes for a combinatorial homeodomain protein. The *a* locus governs cell fusion of haploid cells and filamentous growth of the dikaryon *in vitro* but not *in planta*; the *b* locus is the major determinant of filamentous growth, *in vitro* and *in planta*, and of pathogenicity (reviewed in Banuett, 2002; 2007;Klosterman et al., 2007). In order to complete the life cycle, haploids that fuse must differ

at both *a* and *b* (for example, *a1 b1* + *a2 b2*). The *a* locus has two alleles (*a1* and *a2*) and the *b* locus 25 naturally occurring alleles (*b1*....*b25*), and any combination of different *b* alleles results in an active *b* protein (reviewed in Banuett 2002;2007;Klosterman et al., 2007).

The repertoire of morphologies is expanded by interaction of *U. maydis* with its hosts (Fig. 1). This is evidenced by the formation of a specialized structure for penetration, the appressorium, (see for example, Brachmann et al., 2003; Snetselaar and Mims, 1993), by extensive branching on the leaf surface and inside plant cells (Banuett and Herskowitz, 1996), and by formation of clamp-like structures for nuclear distribution (Scherer et al., 2006). Appressoria, branch formation, and clamp-like structures are not observed in culture. Once tumors are formed, dikaryotic hyphae undergo a discrete developmental program characterized by distinct morphologies (cylindrical cells, spherical cells, and other diverse shapes) that arise upon hyphal fragmentation and lead to formation of the teliospore, a round spore with a specialized cell wall (Fig. 1) (Banuett and Herskowitz, 1996). The distinct cell morphologies present in the plant are not observed *in vitro*. It has thus been proposed that the plant produces signals that trigger fungal differentiation and reorganization of the machinery for polarized growth (Banuett and Herskowitz, 1996). The nature of these signals is not known. Interestingly, the fungus also elicits changes in cell morphology and nuclear position of the host (Banuett and Herskowitz, 1996; Ruiz-Herrera et al., 1999; reviewed in Banuett, 2002). Thus, the interaction of *U. maydis* with its host appears to be a reciprocal process of signal exchange that results in alterations in cell morphology of both host and fungus.

A. The yeast-like cell morphology

The yeast-like cells are elongated with tapered ends (cigar-shaped). They bud once per cell cycle at one of the cell poles. The mechanism by which one of the cell poles is chosen versus the other is not known. Cells are able to bud at new sites at the cell poles or use a previously chosen site for budding as in apiculate yeasts (Jacobs et al., 1994). In some respects this pattern resembles the bipolar budding pattern of *S. cerevisiae*. The *U. maydis* bud grows by tip extension (Banuett and Herskowitz, 2002). There is no phase of isotropic growth, which contrasts with bud growth in *S. cerevisiae* where there is a short phase of polarized growth early in the cell cycle followed by a switch to isotropic growth in the G2/M phase of the cell cycle (reviewed in Pruyne and Bretscher, 2000a,b). Formation of the *U. maydis* bud entails several morphological steps that result in the final shape of the bud (Banuett and Herskowitz, 2002).

B. The filamentous form

In culture, the filamentous form grows by tip extension, as occurs in other filamentous fungi, and divides at the apical cell to produce an actively growing tip cell and a subapical cell devoid of cytoplasm, but little is known about division of the apical cell. *In planta*, most hyphal compartments, not just the tip cell, contain cytoplasm (Banuett and Herskowitz, 1996) and nuclear distribution involves clamp-like structures (Scherer et al., 2006).

C. The Spitzenkörper and filamentous growth

Fungal hyphae exhibit continuous polarized growth; there is no arrest of growth during cytokinesis. Examples of cells that exhibit such highly polarized growth are pollen tubes and root hairs in plants, and neurons in animals.

In filamentous fungi, a fungal-specific phase-contrast opaque body, the Spitzenkörper (apical body), located at or just below the tip of the apical cell, is thought to drive hyphal growth (López-Franco, 1996; Reynaga-Peña et al., 1997). It consists of a heterogeneous population of vesicles surrounding a core that contains polysomes, microtubules, and actin, and is proposed to serve as a supply center for the distribution of vesicles containing materials necessary for

tip extension (reviewed in Bartnicki-García, 2002; Harris et al., 2005; Harris, 2006). The Spitzenkörper can be visualized with the amphiphilic styryl dye FM4-64 (Fischer-Parton et al., 2000). Long-range transport of vesicles from other parts of the hypha to the Spitzenkörper is proposed to occur along microtubules, and delivery from the Spitzenkörper to sites of growth is proposed to occur on actin tracks (reviewed in Harris et al., 2005; Harris, 2006). Both microtubules and actin are necessary for hyphal morphogenesis. Disruption of microtubules, kinesin, dynein, and dynactin results in unstable axes of polarized growth but not in absence of polarized growth *per se*, whereas disruption of actin causes complete loss of cell polarity and results in isotropic growth (Harris et al., 2005; Heath, 2000; Lee et al., 2001; Riquelme et al., 2000; Rupes et al., 1995; Seiler et al., 1999; Torralba et al., 1998a,b; Virag and Griffiths, 2004). Some of these conditions also result in loss or alteration of the position or function of the Spitzenkörper (Crampin et al., 2005; Konzack et al., 2005; Riquelme et al., 2000; Seiler et al., 1999).

There is a dearth of information concerning the molecular composition of the Spitzenkörper. Recent work in different filamentous fungi indicates that formin (a multidomain, actin-nucleating protein), myosin light chain, Sec4 (a Rab GTPase), BemA (homologue of *S. cerevisiae* Bem1), TeaA and TeaR (homologues of *S. pombe* end cell markers Tea1 and Mod5, respectively), and two chitin synthases are components of the Spitzenkörper (see below) (Crampin et al., 2005; Leeder and Turner, 2007; Riquelme et al., 2007; Sharpless and Harris, 2000; Takeshita et al., 2008). The presence of formin suggests that the Spitzenkörper may serve as an actin nucleation center. Little is known about the Spitzenkörper in *U. maydis*.

III. *U. maydis* HOMOLOGUES OF YEAST GENES INVOLVED IN CELL POLARITY AND CELL MORPHOGENESIS

The yeasts, *S. cerevisiae* and *Schizosaccharomyces pombe* have been used extensively in studies of cell polarity and morphogenesis (Chang and Peter, 2003). These studies provide the framework in which to understand these processes in other organisms, in particular the fungi; therefore, we have used these two yeasts as a reference point in our analysis. Below we describe some of the components of the machinery for polarized growth in *S. cerevisiae* (for *S. pombe* see supplementary material), and identify components conserved in *U. maydis*. We describe, where known, their function in *U. maydis*, and in some filamentous fungi.

A. A hierarchy of GTPase modules controls bud site selection and establishment of polarized growth in *S. cerevisiae*

1. Recognition of the spatial landmark—*S. cerevisiae* cells divide by budding and exhibit two budding patterns, axial and bipolar, which are genetically determined by the *MAT* locus. **a** or α cells exhibit the axial budding pattern, in which mother and daughter cells bud immediately adjacent to the previous budding site. **a**/ α cells exhibit a bipolar budding pattern, in which daughters bud distally to the site of their birth, whereas the mother can bud distally or proximally to the previous budding site (reviewed in Casamayor and Snyder, 2002; Chant, 1999). A GTPase module consisting of a Ras-like GTPase Rsr1/Bud1, its guanine nucleotide exchange factor (GEF) Bud5, and its GTPase-activating protein (GAP) Bud2 determine the site of budding in response to a spatial landmark for axial or bipolar budding (reviewed in Casamayor and Snyder, 2002; Chant, 1999). The landmark for axial budding consists of septins, Bud3, Bud4, Axl1, Bud10/Axl2, and for bipolar budding of Bud8, Bud9, Bud7, Rax1, and Rax2. The Rsr1/Bud1 GTPase module is not essential for viability; in its absence cells are still capable of polarized growth but in a random manner (reviewed in Casamayor and Snyder, 2002; Chant, 1999; Park and Bi, 2007).

2. Polarity establishment and the Cdc42 GTPase module—The Rsr1/Bud1 GTPase module interprets the spatial landmark (axial or bipolar) and recruits another GTPase module that consists of the Rho GTPase Cdc42, its guanine nucleotide exchange factor (GEF) Cdc24, and its GTPase-activating proteins (GAPs) Bem3 and Rga1/2 (reviewed in Casamayor and Snyder, 2002; Chant, 1999; Park and Bi, 2007). This module in turn recruits the actin cytoskeleton, which polarizes secretion towards the site of growth (reviewed in Pruyne and Bretscher, 2000a,b).

B. *U. maydis* homologues of landmark proteins and the BUD site selection and polarity establishment GTPase modules

The *U. maydis* genome lacks coding information for homologues of Bud3 and Bud4 (axial landmark), and Bud8 and Bud9 (bipolar landmark) (Table 1), but contains the information for Bud10/Axl2 (axial landmark), and Rax1 and Rax2 (bipolar landmark). Thus, *U. maydis* contains a subset of the genes that code for the axial and bipolar landmark proteins. Their role in budding and cell morphogenesis remains to be determined. Interestingly, in *Ashbya gossypii*, a filamentous fungus closely related to *S. cerevisiae*, AgBud3 appears to act as a landmark for septation (Wendland, 2003). Coding information for components of the Rsr1/Bud1 GTPase module (Rsr1/Bud1, Bud2, and Bud5) and many other recently identified genes required for bipolar budding (*BUD13-32*; Ni and Snyder, 2001) is present in the *U. maydis* genome (Table 1). The function of these genes remains to be determined. In *A. gossypii*, AgRsr1/Bud1 is required for actin organization, for normal hyphal growth and branching, and for position of the polarisome component AgSpa2. Control of the position of a polarisome component by the Rsr1/Bud1 GTPase is a novel function of this GTPase. The *Agrsr1* null mutant is characterized by false branch initiation, pausing and reinitiation of growth resulting in bulges along the hypha, and transient appearance and disappearance of AgSpa2 that correlates with phases of pausing and growth (Bauer et al., 2004). In wild type hyphae, AgSpa2 (a homologue of *S. cerevisiae* Spa2, see below) is present at the hyphal tip continuously. In *Candida albicans*, another fungus closely related to *S. cerevisiae*, CaRsr1/Bud1 defects result in random budding in yeast cells and in defects in germ tube emergence (Yaar et al., 1997).

The *U. maydis* genome contains coding information for homologues of the Rho Cdc42 GTPase module (Cdc42, Cdc24, Bem3, Rga1/2) (Table 1). In addition, *U. maydis*, like other filamentous fungi, contains a homologue of mammalian Rac, another Rho GTPase, which is absent from the genomes of *S. cerevisiae* and *S. pombe*. In mammals, Cdc42 and Rac1 play partially overlapping roles in morphogenesis, cell division, and migration (Jaffe and Hall, 2005). Likewise in filamentous fungi, Cdc42 and Rac appear to have partially overlapping functions in control of cell polarity (see for example, Bassilana and Arkowitz, 2006; Boyce et al., 2001, 2003; Chen and Dickman, 2004, 2006; Rolke and Tudzynski, 2008; Scheffer et al., 2005; Virag et al., 2007).

In *U. maydis*, Cdc42 is required for cell separation but not for polarized growth, and is not essential for viability (Mahlert et al., 2006; F. Banuett, unpublished results), whereas UmRac1 is required for normal cell morphology of the yeast-like cell and for positioning of the septum (Mahlert et al., 2006). *Umc42* null mutants form chains of cells that exhibit wild type morphology, separated by a single septum (Mahlert et al., 2006). In wild type cells, there are two septa prior to cell separation, one on the mother side and the other on the bud side. Cell separation occurs in the region between these two septa, the fragmentation zone (Banuett and Herskowitz, 2002; O'Donnell and McLaughlin, 1984; Weinzierl et al., 2002). In *S. cerevisiae* *CDC42* is essential for viability and for polarity establishment: *cdc42^{ts}* and *cdc24* mutants arrest as large unbudded, multinucleate cells (reviewed in Pruyne and Bretscher, 2000a,b). Thus, *U. maydis* and *S. cerevisiae* use Cdc42 differently, a theme repeated in other filamentous fungi (see below).

The importance of Rac1 in polarized growth is supported by the following observations: expression of an activated allele of Rac1 results in formation of balloon-like structures at the tips of yeast-like cells; and *Umrac1* null mutants exhibit elongated abnormal cells with rounded ends and a septum in the middle; wild type cells, in contrast, have tapered ends (Mahlert et al., 2006). UmRac1 is not required for cell viability, but depletion of UmRac1 in a strain lacking *Umc42* is lethal: cells arrest as chains of enlarged, aberrant cells, with a single nucleus, and delocalized cell wall material (Mahlert et al., 2006). This observation suggests that Cdc42 and Rac1 share a common target. Rac1 is also necessary for the b-dependent transition from yeast-like to filamentous form: induction of *Umrac1* expression in haploid cells (haploids normally lack an active b protein) causes a transition from yeast-like to filamentous form. UmRac1 is thus necessary and sufficient for the dimorphic switch that results in filament formation (Mahlert et al., 2006). The GEF and GAP regulators of the UmRac1 GTPase remain to be identified.

In *Aspergillus nidulans* Cdc42 is required for hyphal morphogenesis, but it is not required for localization of polarisome components, the Spitzenkörper, or organization of actin, and neither is AnRac1 required for localization of polarisome components or the Spitzenkörper (Virag et al., 2007). In contrast, *S. cerevisiae* Cdc42 is required for localization of polarisome components and actin organization (reviewed in Chang and Peter, 2003; Pruyne and Bretscher, 2000a, b). AnCdc42 and AnRac likely share a common target because double *Anrac1 Ancdc42* mutants are synthetic lethal: the double mutants are unable to polarize and lyse as attached rounded cells separated by septa (Virag et al., 2007). In *Penicillium marneffeii*, Rac is required for normal organization of the actin cytoskeleton. Rac regulates polarized growth of the hypha in concert with Cdc42 but the specific steps controlled by these Rho GTPases differ (Boyce et al., 2001, 2003). The synthetic lethality of *cdc42* and *rac1* in *U. maydis* and other filamentous fungi supports the notion that these Rho GTPases play partially overlapping roles in control of cell polarity.

C. *U. maydis* homologues of effectors of Cdc42

In *S. cerevisiae*, downstream effectors of Cdc42 include the p21-activated kinases (PAKs) Cla4, Ste20, and Skm1, and two proteins of unknown function, Gic1 and Gic2 (reviewed in Pruyne and Bretscher, 2000a, b). Ste20 and Cla4 are important for the Cdc42-dependent reorganization of the actin cytoskeleton during the cell cycle. The *U. maydis* genome contains coding information for three Ste20-like kinases (Table 1): Smu1, Cla4, and Don1, but not for Gic1 and Gic2. Smu1 and Cla4 contain the CRIB domain present in p21-activated kinases (PAKs), whereas Don1 lacks this domain, and thus belongs to the germinal center (GCK) subfamily of Ste20-like kinases (Leveleki et al., 2004; Smith et al., 2004). (For further details on Smu1, see the article by García-Pedrajas et al. in this issue). Both UmDon1 and UmCla4 play a role in cytokinesis (Leveleki et al., 2004), just as Cla4 and Ste20 do in *S. cerevisiae*. Two observations support the idea that UmCla4 is likely to be a downstream effector of UmRac1: both $\Delta cla4$ and $\Delta rac1$ mutants have a similar phenotype, and they show yeast two-hybrid interactions. Other effectors of Rac1 are likely to exist because $\Delta cla4$ does not suppress the lethality observed upon expression of an activated allele of Rac1 (Mahlert et al., 2006).

D. *U. maydis* homologues of other Rho proteins

The Rho subfamily of GTPases includes Rho, Rac, Cdc42, and others. They act as molecular switches that play important roles in cell polarity, morphogenesis, and cell division (Jaffe and Hall, 2005). *S. cerevisiae* contains one Cdc42 (described above) and five Rho proteins, Rho1-5. Rho1, an essential gene for viability, is required for polarity establishment. Rho3 and Rho4 are also required for polarized growth. The signal from different Rho proteins (Rho1, Rho3, Rho4, and Cdc42) converges on the formin Bni1, a polarisome component, to control actin organization (see below) (Dong et al., 2003; reviewed in Pruyne and Bretscher, 2000a,b). In

addition, Rho1 controls bud morphogenesis via two different outputs: as a catalytic subunit of β -glucan synthase, the enzyme that synthesizes the major component of the cell wall, and as an activator of protein kinase C (Pkc1) that controls the cell wall integrity signal transduction pathway (reviewed in Banuett, 1998; Cabib et al., 1998; Pruyne and Bretscher, 2000a,b). The regulators of Rho1 include two redundant GEFs, Rom1 and Rom2, and two GAPs, Sac7 and Bem3. The *U. maydis* genome contains coding information for three Rho proteins, Rho1, Rho2, and Rho3, and for homologues of Rho1 regulators (Table 1). Their function has not been reported. Rho proteins in other filamentous fungi are required for polarized growth and it is likely that this function will be conserved in *U. maydis*. In *A. gossypii*, *rho3* mutants exhibit swelling of hyphal tips indicative of isotropic growth, and *rho1* null mutants exhibit lysis of cells in a colony (Wendland and Philippsen, 2001). In *A. nidulans*, RhoA, a homologue of *S. cerevisiae* Rho1, is necessary for polarized growth, branching pattern, and cell wall deposition (Guest et al., 2004).

E. *U. maydis* homologues of polarisome components

In *S. cerevisiae*, the polarity determining proteins, Spa2, Pea2, Bud6/Aip3, and Bni1, assemble at the bud tip and function as a scaffold for the assembly of other proteins. Mutants lacking either of these proteins exhibit defects in actin organization, apical growth, and have widened necks (reviewed in Pruyne and Bretscher, 2000a,b). The polarisome links Rho GTPases and actin cable assembly through the formin Bni1. Formins are multidomain proteins required for maintenance of cell polarity, cytokinesis, and actin organization, and are central to how inputs from Rho GTPases are transmitted to actin organization (Chang and Peter, 2003; Evangelista et al., 2003; Wallar and Alberts, 2003). Bni1 binds profilin, an actin-monomer binding protein, and has been shown to nucleate actin cables. Bud6 is important for the Bni1 actin-nucleation function (Pruyne et al., 2002; Sagot et al., 2001). Localization of Bni1 to the bud tip requires Spa2 and Pea2. Some polarisome components also localize to the mother-bud neck region during cytokinesis to participate in formation of the actomyosin ring and the septum (reviewed in Pruyne and Bretscher, 2000a,b).

The *U. maydis* genome contains coding information for homologues of polarisome components Bud6, Spa2, and two formins, UmSepA and UmDia, but not of Pea2 (Table 1). The role of these proteins in cell polarity of the yeast-like and filamentous form in *U. maydis* remains to be determined. Recent studies in *A. gossypii*, *A. nidulans*, and *C. albicans* (Crampin et al., 2005; Knechtle et al., 2003; Virag and Harris, 2006) are providing insights about the role of the polarisome complex in polarized growth of the hypha. In *A. nidulans*, homologues of Spa2, Bud6/Aip3, and Bni1 are necessary for maintaining the axis of polarized growth: mutations in either component result in dichotomous branching (branching at the hyphal tip), which is normally not observed in wild type hyphae (Sharpless and Harris, 2002; Virag and Harris, 2006). SpaA::GFP localizes to the hyphal tip, overlapping to some extent the localization of the Spitzenkörper, consistent with a role at the hyphal tip, possibly as a component of the Spitzenkörper (Virag and Harris, 2006). SpaA is not required for localization of the formin SepA (Virag and Harris, 2006), whereas *S. cerevisiae* Spa2 is required for localization of the formin Bni1 (reviewed in Pruyne and Bretscher, 2000a,b). BudA, the *A. nidulans* homologue of Bud6/Aip3, localizes to sites of septum formation. The *AnbudA* mutant phenotype is consistent with a role in maintenance of the axis of polarized growth and determining the area of growth. *AnbudA* null mutants exhibit reduced hyphal growth rate, increased hyphal diameter and nuclei per hyphal segment, dichotomous branching, and increased branching frequency, and potato-shaped germings (Virag and Harris, 2006). AnSepA is required for septum formation, for maintenance of the axis of polarized growth, and determining the area of growth, and normal organization of actin at the hyphal tips and septa. SepA null mutants exhibit wider hyphal diameter, dichotomous branching, and lack septa. SepA::GFP localizes to hyphal tips, coincident with localization of the Spitzenkörper, and also to septa (Sharpless and Harris,

2002). This localization led to the hypothesis that the Spitzenkörper acts as an actin-nucleating center (Sharpless and Harris, 2002).

In *A. gossypii*, the Spa2 homologue, which localizes permanently to the hyphal tip and to sites of branch initiation, appears to determine area of growth, normal growth rate, and branching density (Knechtle et al., 2003). *AgBNII* is necessary for establishment of polarized growth and for actin organization. Disruption of *AgBNII* results in formation of giant potato-shaped cells lacking actin cables and also in the inability to form hyphae (Schmitz et al., 2006). *AgBni1* localizes to hyphal tips. A constitutively active *AgBni1* results in dichotomous hyphal branching in young hyphae, a type of branching normally restricted to mature hyphae (Schmitz et al., 2006). The localization of these proteins at the hyphal tip supports a role in determining polarized growth.

In *C. albicans*, *CaBni1* and *CaMlc1* (myosin light chain) colocalize with the Spitzenkörper, whereas *Spa2* and *Bud6/Aip3* localize mostly as a cap at the hyphal tip that overlays the Spitzenkörper (Crampin et al., 2005). Thus, in *C. albicans* it seems that polarized growth of hyphae is determined by two separate entities, the Spitzenkörper and the polarisome, whereas in pseudohyphae and yeast-like cells, the polarisome is responsible for polarized growth; there is no Spitzenkörper in these cells.

F. *U. maydis* homologues of septins

Septins are GTP binding proteins that are conserved from fungi to mammals and play a role in cytokinesis and other cell processes (Gladfelter et al., 2001; Longtine and Bi, 2003; Pan et al., 2007). In *S. cerevisiae*, septins form filaments at the mother-bud neck region, arranged longitudinally (along the main cell axis) in an hour-glass shape or as horizontal rings in a cell cycle-dependent manner (Gladfelter et al., 2001). Assembly of septins requires Cdc42, polarisome components, the PAK kinase, Cla4, and the actin cytoskeleton (Kadota et al., 2004). Septins are proposed to serve a scaffold role and recruit other proteins: bud site selection landmarks, components of the actomyosin contractile ring (CAR), chitin synthases, and other proteins (Gladfelter et al., 2001; Longtine and Bi, 2003). Septins also act as diffusion barriers for integral membrane proteins and have been proposed to serve as organizers of cortical domains of the cell in addition to their scaffold role (Barral et al., 2000; Takizawa et al., 2000).

S. cerevisiae contains 7 septins, Cdc3, Cdc10, Cdc11, Cdc12, Sep7, Spr4, Spr28; the latter two are meiosis-specific and required with other septins during spore maturation. The *U. maydis* genome contains coding information for homologues of *S. cerevisiae* Cdc3, Cdc10, Cdc11, and Cdc12 (Table 1), whereas in other filamentous fungi there appear to be at least five putative septins (see Borkovich et al., 2004). *U. maydis* Sep3, a homologue of Cdc11, is required for normal cell morphogenesis (Boyce et al., 2005). Haploid Sep3 mutants exhibit aberrant cell morphology: the cells are “lemon drop-shaped”, with a septum in the middle, become multinucleated, and have altered chitin deposition. Sep3 is also required for normal morphology of the promycelium (a short filament produced upon germination and meiosis of the teliospore; Christensen, 1963), and the basidiospores (the haploid cells produced on the promycelium by mitosis of the four primary meiotic products). Genetic analysis indicates that Sep3 acts in the cAMP signaling pathway (Boyce et al., 2005). The role of other septins in *U. maydis* remains to be uncovered. In *A. nidulans*, *AspB*, a homologue of *S. cerevisiae* Cdc3, is hypothesized to act as a marker of branch formation. It localizes premitotically as a ring to sites of branching and secondary germ tube emergence. It also localizes postmitotically to sites of septation (Westfall and Momany, 2002).

G. *U. maydis* homologues of the exocyst complex

In *S. cerevisiae*, the exocyst is a protein complex required for exocytosis and consists of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 (TerBush et al., 1996). It is localized to the bud tip during polarized growth and to the neck region during cytokinesis, and is involved in fusion and docking of vesicles transported on actin cables. The Sec3 protein acts as a spatial landmark for the assembly of the exocyst complex (Finger et al., 1998). The assembly of the different protein complexes described above provides a temporal and spatial link between bud site selection with organization of the cytoskeleton, and secretion resulting in cell growth.

The *U. maydis* genome contains coding information for all the components of the exocyst complex (Table 1) but little is known of their role in polarized growth and cytokinesis. In *A. nidulans*, SecC, a homologue of *S. cerevisiae* Sec3, accumulates at the plasma membrane anterior to the Spitzenkörper and may function as a landmark for secretion (Taheri-Talesh et al., 2008).

IV. THE CYTOSKELETON

The cytoskeleton plays an important role in cell morphogenesis and polarized growth in diverse organisms. In *U. maydis*, both actin and microtubules are required for cell morphogenesis, as in other filamentous fungi.

A. The actin cytoskeleton

The actin cytoskeleton in *U. maydis* consists of three structures: actin patches, actin cables, and an actin ring as observed in other fungi. The actin cytoskeleton is highly polarized throughout the cell cycle: actin patches concentrate at the presumptive bud site and at the tip of the growing bud, and actin cables polarize towards the actin patches and extend into the mother cell (Banuett and Herskowitz, 2002). During cytokinesis, actin forms a ring in the neck region. Actin patches persist at the growing end during this process. After cell division is complete, both cell ends contain actin patches, with one end having a higher concentration than the other. This distribution of actin is consistent with highly polarized growth at the tip of the cell throughout the cell cycle. There is no evidence for a disorganized actin cytoskeleton at any stage of the cell cycle as occurs during isotropic growth in *S. cerevisiae*, consistent with observations that there is no isotropic growth during bud morphogenesis in *U. maydis* (Banuett and Herskowitz, 2002). *U. maydis* hyphae contain a prominent actin cap at the tip and actin cables polarize towards the tip, as observed in other filamentous fungi (Banuett and Herskowitz, 2002).

1. *U. maydis* homologues of components of the actin cytoskeleton—In *S. cerevisiae*, the actin cytoskeleton is involved in polarized secretion. Actin cables serve as tracks on which vesicles move on myosin motors towards sites of active growth and secretion (the bud tip during polarized growth and the neck region during cytokinesis) (Karpova et al., 2000; Pruyne and Bretscher, 2000a,b). All components involved in actin assembly and function in *S. cerevisiae* appear to be conserved in the *U. maydis* genome (Table 1). For example, genes coding for the Arp2/3 complex (Winter et al., 1999), responsible for nucleation of branched actin structures, are present in the *U. maydis* genome, as well as genes coding for homologues of Bee1/Las17, the activator of Arp2/3 (a homologue of the mammalian Wiskott-Aldrich syndrome protein WASP), and Vrp1 (homologue of mammalian Wip, Wiskott-Aldrich syndrome protein interacting-protein). In *S. cerevisiae*, Arp2/3, Bee1/Las17, and Vrp1 localize to actin patches and form an actin cap during early bud morphogenesis (reviewed in Pruyne and Bretscher, 2000a,b). The *S. cerevisiae* genome contains two tropomyosin isoforms, Tpm1 and Tpm2. These proteins stabilize actin cables (reviewed in Pruyne and Bretscher, 2000a,b). The *U. maydis* genome contains a single tropomyosin gene, and the putative protein is similar

to *S. cerevisiae* Tpm2. The function of tropomyosin in *U. maydis* remains to be determined. The *U. maydis* genome contains coding information for homologues of proteins such as actin binding protein (Abp1), coronin (Crn1), cofilin (Cof1), profilin (Pfy1), fimbrin (Sac6), Pan1, Prk1, Srv2, Sla1 and Sla2, some of which are actin patch components (Table 1). The function of these proteins in *U. maydis* has not been reported. (See Moseley and Goode, 2006, for a review on the function of these proteins in *S. cerevisiae*).

2. *U. maydis* homologues of myosins—As in other filamentous fungi, the *U. maydis* genome contains coding information for 4 myosins: a class I (Myo1), a class II (Myo2), a class V (Myo5), and a class XVII (myosin-chitin synthase; Csm1) (Table 1) (see Hodge and Cope, 2000, for myosin classification). Myosin-chitin synthase contains an amino-terminal myosin domain and a chitin synthase domain at the carboxy terminus. It is specific to the filamentous fungi; the genomes of *S. cerevisiae* or *S. pombe* lack this information. In the yeast-like cell, *Ummyo5* is required for cell morphogenesis and cell separation: *Ummyo5* null mutants are viable and exhibit slow growth and formation of clusters of shorter and wider cells, which fail to separate (Weber et al., 2003). Young *myo5* mutant cells still exhibit polarized growth and polarized chitin at the cell tip, whereas older cells lose polarity and become aberrantly shaped (Weber et al., 2003). In the hypha, *Ummyo5* appears to be required for the axis of polarized growth and to determine the area of growth: hyphae deficient in Myo5 are wider in diameter and exhibit an irregular growth pattern (spiral-like), and contain irregular deposition of chitin along the hyphal length (Weber et al., 2003). Myo5::GFP localizes to the incipient bud site, the bud tip in cells with small buds, and becomes more dispersed in large-budded cells; in hyphae, it localizes to the tip, consistent with a role at the cell tip (Weber et al., 2003). The function of Myo1 and Myo2 has not been reported. (See below for role of Csm1).

B. The microtubule cytoskeleton

In many eukaryotes, the microtubule cytoskeleton is required for polarized growth, transport of organelles and vesicles, in addition to its role in spindle assembly and function.

The microtubule cytoskeleton in *U. maydis* consists of an extensive cytoplasmic array throughout interphase that disassembles in late G2 when a short intranuclear spindle is formed and astral microtubules are assembled from the spindle pole bodies (Banuett and Herskowitz, 2002; Steinberg et al., 2001; Straube et al., 2003). These astral microtubules appear to be involved in nuclear movements to the bud, where the nucleus divides, and then after nuclear division, when one of the nuclei migrates to the mother cell (Banuett and Herskowitz, 2002; Fink et al., 2006; Steinberg et al., 2001). The organization of the microtubule cytoskeleton in *U. maydis* is more complex than that in *S. cerevisiae*, and is reminiscent of that observed in *S. pombe*. In *U. maydis* yeast-like cells, nucleus-associated spindle pole bodies (SPBs) nucleate astral microtubules, and cytoplasmic microtubule organizing centers (MTOCs), assembled at the base of the bud early during bud morphogenesis, nucleate microtubules towards the bud and mother cell (Straube et al., 2003). The yeast-like cell contains additional motile cytoplasmic MTOCs (Fink and Steinberg, 2006; Straube et al., 2003). In the hypha, the majority of MTs are oriented with their plus ends towards the growing tip; some MTs are oriented with their plus ends towards the basal end of the apical cell (Schuchart et al., 2005).

The microtubule cytoskeleton is required for normal cell morphogenesis in *U. maydis* (Steinberg et al., 2001), just as it is in *S. pombe* (Sawin and Nurse, 1998; reviewed in Chang, 2001). In contrast, *S. cerevisiae* MTs play no role in cell morphogenesis. *U. maydis* thus resembles *S. pombe* in the organization of the microtubule cytoskeleton and in the requirement of the microtubule cytoskeleton for cell morphogenesis and for determining the axis of polarized growth. *U. maydis* may thus combine strategies used by *S. cerevisiae* and *S. pombe* in novel ways to establish and maintain polarized growth and to generate cell shape.

1. *U. maydis* homologues of tubulins—The *U. maydis* genome contains coding information for an α tubulin (*tub1*), two β tubulins, and a γ tubulin (*tub2*) (Table 1). Both α and γ tubulin genes have been characterized and shown to be essential for viability (Steinberg et al., 2001; Straube et al., 2003). γ tubulin is found at the SPBs and also in the cytoplasmic and polar MTOCs and its presence in these structures varies in a cell cycle dependent manner (Straube et al., 2003). α tubulin is required for cell morphology and polarized growth. One β tubulin gene was cloned, but its role in cell polarity and cell morphogenesis was not reported (Gold et al., 1994).

2. *U. maydis* homologues of microtubule-plus-end-associated proteins (+TIPS)

—+TIPs are a highly diverse group of proteins that includes MT-dependent motors and nonmotor proteins: CLIP170 (ScBik1), Dynactin (Sc Jnm1, Nip100, Arp1, Arp10), EB1 (Sc Bim1), CLASPS (Sc Stu1), LIS1 (Sc Pac1), Dynein (Sc Dhc1), APC (Sc Kar9), and others (Adkhmanova and Hoogenraad, 2005).

Dynein and dynactin: Cytoplasmic dynein is a multisubunit complex with a MT minus-end directed motor activity. In *U. maydis*, as in other fungi, there is a single cytoplasmic dynein. The heavy chain of cytoplasmic dynein is encoded by two unlinked genes: *dyn1* codes for the putative ATPase region, and *dyn2* codes for the microtubule binding region (Straube et al., 2001). Dyn1 and Dyn2 polypeptides interact to form the active protein. Both genes are essential. Analysis of conditional mutations indicates that dynein is required for cell morphogenesis, nuclear migration, microtubule organization, retrograde endosomal transport, and organization of the endoplasmic reticulum (Straube et al., 2001; Wedlich-Soldner et al., 2002). Mutant cells in either *dyn1* or *dyn2* lose polarity (become rounded), contain multiple nuclei that cluster in the aberrantly shaped cells and microtubules that become disorganized, and develop long projections that exhibit polarized growth (Straube et al., 2001).

Dynactin is a multiprotein complex required for activation of cytoplasmic dynein-mediated vesicular transport. It consists of at least ten polypeptides in most eukaryotes; in yeast, four dynactin subunits have been identified (see above). The p150^{Glued} subunit is the largest and interacts with the 74-kDa dynein intermediate chain. Other subunits include the actin-related protein Arp1/centractin (45 kDa); the 50 kDa/dynamitin subunit; dynactin p62, dynactin p27, dynactin, p25, dynactin p24/p22, and CapZ (reviewed in Schroer, 2004; Xiang and Plaman, 2003). In *S. cerevisiae* the dynactin complex is required for nuclear migration (Lee et al., 2003; Sheeman et al., 2003). In *Neurospora crassa*, it is also required for nuclear migration, in addition to a role in determining the axis of polarized growth, and position of the Spitzenkörper (Plaman et al., 1994; Riquelme et al., 2000; Seiler et al., 1999). The *U. maydis* genome contains coding information for homologues of components of the *S. cerevisiae* dynactin complex (Table 1), and of other dynactin components found in filamentous fungi and other eukaryotes (data not shown), and other +TIPs (Table 1). UmDya1 (the homologue of p150^{Glued}), UmClip1 (CLIP170 homologue), UmLis1 (LIS1/NudF homologue), and UmPeb1 (homologue of EB1) localize to microtubule plus ends (Lenz et al., 2006; Straube et al., 2003). UmDya1 is required for localization of dynein to microtubule plus ends. UmLis1/NudF is required for activation of dynein and this activation is necessary for retrograde endosomal movement. UmClip1 is not required for retrograde endosomal movement or binding of dynein to microtubule plus ends (Lenz et al., 2006). In *S. cerevisiae*, Kar9 and Pac1/LIS1 are required for orientation of the mitotic spindles and nuclear migration (Lee et al., 2003; Miller and Rose, 1998; Sheeman et al., 2003). The *U. maydis* genome does not contain coding information for a Kar9 homologue.

3. *U. maydis* homologues of kinesins—In filamentous fungi, as in many other eukaryotes, kinesins are involved in long-distance transport of vesicles. *S. cerevisiae* contains 6 kinesins whereas filamentous fungi contain 10–12 kinesins (see Borkovich et al., 2004; Xiang

and Plamann, 2003). The *U. maydis* genome contains coding information for 10 kinesins (Kin1, Kin3, Kin4, Kin6, Kin7a, Kin7b, Kin8, Kin9, and Kin14), some of which are more closely related to their metazoan counterparts than to the ascomycetous fungi (Schuchardt et al., 2005). Genetic analysis has shown that all except Kin1 and Kin3 are dispensable for hyphal growth. Null *kin1* or *kin3* mutants exhibit bipolar growth and short hyphae. Double *kin1 kin3* null mutants exhibit the same phenotype as the single mutants, indicating that they likely act in the same pathway (Schuchardt et al., 2005). Interestingly, *kin1* and *kin3* mutations exacerbate the mutant phenotype conferred by a *myo5^{ts}* mutation. The residual polar growth in the *myo5^{ts}* mutant is completely abolished resulting in clusters of misshapen cells that exhibit no polar growth. This synthetic interaction suggests that Kin1 and Kin3 share a common target with Myo5 for polarized growth. Kin1, Kin3, and Myo5 all accumulate at the hyphal apex, the region where the Spitzenkörper localizes; however, localization of the Spitzenkörper in these studies was not reported.

V. *U. maydis* homologues of chitin synthases

Integrity of the cell wall is crucial for maintenance of cell shape (Latge, 2007; Klis et al., 2006). Chitin and β -glucan are the major components of fungal cell walls (see article by Ruiz-Herrera in this issue). The *U. maydis* genome contains 7 genes that code for chitin synthases (Chs1, Chs2, Chs3, Chs4, Chs5, Chs6, Chs7) and one gene for a hybrid myosin-chitin synthase protein (Mcs1; see above) (Garcerá-Teruel et al., 2006; Gold and Kronstad, 1994; Weber et al., 2006). Deletion analysis of single genes indicates that *Umchs1*, *Umchs2*, *Umchs3*, *Umchs4* are not required for morphology of the yeast-like cell or the hypha, whereas Δ *chs6* results in cells with wider diameter, and Δ *mcs1* cells exhibit slightly wider cell diameter and a ballooning of the bud tip, indicative of a role in cell morphogenesis. The *Umchs5* or *Umchs7* null mutants exhibit a more pronounced phenotype indicative of a role in cell morphology and cell separation, respectively: Δ *chs5* mutant cells are more elongated than wild type cells, grow irregularly, and have a neck region that is not clearly defined, whereas Δ *chs7* mutant cells have a cell separation defect but the cells have normal cell morphology, though they are shorter, and in some cases, lose their normal cell shape (Weber et al., 2006). Six of the *U. maydis* chitin synthases (Chs3-Ch7 and Mcs1) localize to the septal region, and four of them (Chs5, Chs6, Chs7, and Mcs1) also localize to the hyphal tip. Despite the localization of Chs5, 6, 7, and Mcs1 to the hyphal tip, they do not seem to be required for hyphal morphology in culture (Weber et al., 2006; see also Ruiz-Herrera et al., 2006). It is likely that the chitin synthases have partially overlapping functions and are able to substitute for each other when one or two are absent. Deletion of multiple chitin synthases that localize to the hyphal tip will likely be informative (Weber et al., 2006).

VI. FUTURE DIRECTIONS

Our analysis indicates that most of the components of the *S. cerevisiae* machinery for cell polarity and cell morphogenesis, and the cytoskeleton are conserved in *U. maydis*. Because this analysis was based on *S. cerevisiae* and *S. pombe* genes, other components of the cell polarity machinery specific to *Ustilago maydis* or the Basidiomycetes remain unidentified. Sequence comparisons with other filamentous fungi, where additional genes required for polarized growth have been identified, in combination with genetic screens should prove useful in obtaining a more comprehensive view of the machinery for cell polarity and cell morphogenesis.

The challenge for the future is not only to identify the machinery for cell polarity, but also to understand how the components of the machinery are assembled and rearranged during polarized growth, and how these assemblies generate the diversity of forms that characterize the life cycle of *Ustilago maydis*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Different morphologies in the life cycle of *U. maydis*

U. maydis exhibits two basic morphologies, a yeast-like and a filamentous form. Interaction with the plant increases the repertoire of morphologies observed suggesting that plant signals play a role in reorganization of the machinery for cell polarity, cell morphogenesis, and the cytoskeleton. **Panel A.** Different stages of budding of haploid yeast-like cells. **Panel B.** Dikaryotic filaments in culture. No branching is observed. **Panels C, D, E, and F.** Dikaryotic filaments growing in the plant. **Panel C** shows a branch (arrowhead). **Panel D** shows a clamp-like structure (arrowhead) (see Scherer et al., 2006). **Panels E and F** show multiple branches (arrowheads in Panel F). The cell wall of the plant cell is indicated an arrow. **Panel G.** Cylindrical cells released upon hyphal fragmentation within the tumors during teliospore formation. **Panel H.** The cylindrical cells undergo morphological changes during teliospore formation. The cells in Panels G and H are normally embedded in a mucilaginous matrix, which may provide osmotic support during the cell wall remodelling events occurring during these morphological transitions (Banuett and Herskowitz, 1996). **Inset (I).** Mature teliospores with echinulate cell wall. The teliospores give rise to haploid yeast-like cells upon meiosis. The scale bar in Panel A is 5 μ m, and in Panels B, C, D, E, F, and H, 50 μ m.

***U. maydis* homologues of the *S. cerevisiae* machinery for cell polarity, cell morphogenesis, and the cytoskeleton**

S. cerevisiae genes involved in cell polarity, cell morphogenesis, and the cytoskeleton (column 2) were used to probe the *U. maydis* genome with wuBlast at the MIPS *Ustilago* database MUMDB (<http://mips.gsf.de/genre/proj/ustilago/>), and the *Ustilago maydis* Database at the Broad Institute (http://www.broad.mit.edu/annotation/genome/ustilago_maydis/Home.html). We also used Blast at NCBI (<http://www.ncbi.nlm.nih.gov/blast>) (Altschul et al., 1997). The genes identified in *U. maydis* were then used to search for homologues in the Basidiomycota and Ascomycota, and in other eukaryotes using the SIMAP tool at MIPS (see Fig.1S, supplementary material). The source of *S. cerevisiae* gene products was Drees et al., 2001; Pruyne and Bretscher, 2000; and GO annotation at the *Saccharomyces* genome database (<http://www.yeastgenome.org/>). The systematic name for *S. cerevisiae* genes (column 3) was obtained from (<http://www.yeastgenome.org/>). *U. maydis* gene numbers (column 4) were obtained from (<http://mips.gsf.de/genre/proj/ustilago/>) after BLAST analysis. E values (column 5) indicated are as obtained from wuBlast analysis at MIPS. Other parameters were also used in our Blast analysis, in particular in the comparison across different fungal groups and other eukaryotes (data not shown but available upon request). The last column provides the function of the *S. cerevisiae* genes (<http://www.yeastgenome.org/>). The list of genes in this Table is not comprehensive; it does not include many genes involved in cytokinesis, cell separation, secretion, and cell cycle that were used in the analysis shown in Figs 1S (supplementary material).

CELL POLARITY/CELL MORPHOGENESIS					
Sc gene	Systematic name	<i>U. maydis</i> MIPS#	E value	Function in <i>S. cerevisiae</i>	
1 <i>BAG7</i>	YOR134W	02902	3.3e-48	RhoGAP: functionally related to Sac7	
2 <i>BEM1</i>	YBR200W	10934	3.7e-44	Establishment of cell polarity. Scaffold for different protein complexes	
3 <i>BEM2</i>	YER155C	01161	3.4e-42	RhoGAP	
4 <i>BEM3</i>	YPL115C	11852	8.9e-24	GAP for Cdc42 and Rho1	
5 <i>BEM4</i>	YPL161C			Interacts with Cdc42 and Rho1. Bud emergence.	
6 <i>BNI4</i>	YNL233W		no hits	Required for localization of CHSIII to bud neck	
7 <i>BOI1</i>	YBL085W	03037	1.7e-23	Bem1-binding protein. Polarized growth	
8 <i>BOI2</i>	YER114C	03037	1.8e-19	Bem1-binding protein. Polarized growth	
9 <i>BUD1/RSR1</i>	YGR152C	11909	7.8e-49	Bud site selection. RAS superfamily protein	
10 <i>BUD2</i>	YKL092C	01282	6.9e-10	Bud site selection. GAP for Bud1/Rsr1	
11 <i>BUD3</i>	YCL014W		no hits	Bud site selection. Axial landmark	
12 <i>BUD4</i>	YJRO92W		no hits	Bud site selection. Axial landmark	
13 <i>BUD5</i>	YCKO38C	11476	1.3e-12	Bud site selection. GEF for Bud1/Rsr1	
14 <i>BUD6/AIP3</i>	YLR319C	11657	1.6e-70	Polarisome component: interacts with formin and actin	
15 <i>BUD7</i>	YOR295W	04959	5.2e-62	Export of Chs3 from Golgi to plasma membrane. Bipolar budding.	
16 <i>BUD8</i>	YLR353W		no hits	Bud site selection. Bipolar landmark	
17 <i>BUD9</i>	YGR041W		no hits	Bud site selection. Bipolar landmark	
18 <i>BUD10/AXI2</i>	YIL140W	01289	2.8e-24	Bud site selection	
19 <i>BUD13</i>	YGL174W	00191	1.1e-10	Bud site selection. Bipolar budding.	
20 <i>BUD14</i>	YAR014C	01012	3.4e-14	Bud site selection. Bipolar budding.	
21 <i>BUD15/EDE1</i>	YBL047C	05533	4.8e-61	Bud site selection. Bipolar budding.	
22 <i>BUD16</i>	YEL029C	10310	1.1e-42	Bud site selection. Bipolar budding.	
23 <i>BUD17</i>	YNR027W	10310	1.1e-41	Bud site selection. Bipolar budding.	
24 <i>BUD20</i>	YLR074C	04625	2.5e-11	Bud site selection. Bipolar budding.	
25 <i>BUD21</i>	YOR078W		no hits	Bud site selection. Bipolar budding.	
26 <i>BUD22</i>	YMR014W	01709	4.8e-13	Bud site selection. Bipolar budding.	
27 <i>BUD23</i>	YCR047C	01551	8.4e-68	Bud site selection. Bipolar budding.	
28 <i>BUD25</i>	YER014C-A		no hits	Bud site selection. Bipolar budding.	
29 <i>BUD27</i>	YFL023W		no hits	Bud site selection. Bipolar budding.	
30 <i>BUD31</i>	YCR063W	10057	1.0e-46	Bud site selection. Bipolar budding.	
31 <i>BUD32</i>	YGR262C	12089	2.3e-20	Bud site selection. Bipolar budding.	
32 <i>CDC3</i>	YLR314C	10503	1.1e-117	Septin	

CELL POLARITY/CELL MORPHOGENESIS					
Sc gene	Systematic name	<i>U. maydis</i> MIPS#	E value	Function in <i>S. cerevisiae</i>	
33	<i>CDC10</i>	YCR002C	1.6e-96	Septin	
34	<i>CDC11</i>	YJR076C	03449	Septin	
35	<i>CDC12</i>	YHR107C	03599	Septin	
36	<i>CDC24</i>	YAL041W	02422	GEF for Cdc42. Polarity establishment	
37	<i>CDC42</i>	YLR229C	00295	Small GTP-binding protein of RAS superfamily. Polarity establishment and maintenance.	
38	<i>CHS1</i>	YNL192W	10117	chitin synthase I	
39	<i>CHS2</i>	YBR038W	10120	chitin synthase II	
40	<i>CHS3</i>	YBR023C	10277.2	chitin synthase III	
41	<i>CHS4/SKT5</i>	YBL061C	10641	Activator of chitin synthase III	
42	<i>CHS5</i>	YLR330W	02840	Localization of chitin synthase III	
43	<i>CHS6</i>	YJL099W	04959	Transport of chitin synthase III from Golgi to plasma membrane	
44	<i>CHS7</i>	YHR142W	11089	Export of Chs3 from ER	
45	<i>CKA1</i>	YIL035C	01180	Casein kinase II, alpha subunit	
46	<i>CKA2</i>	YOR061W	01180	Casein kinase II, alpha subunit	
47	<i>CKB1</i>	YGL019W	01094	Casein kinase II, beta subunit	
48	<i>CKB2</i>	YOR039W	06107	Casein kinase II, beta subunit	
49	<i>CLA4</i>	YNL298W	10145	PAK family kinase. Cdc42 effector	
50	<i>CLC1</i>	YGR167W	01316	Clathrin light chain	
51	<i>CMD1</i>	YBR109C	03910	Calmodulin	
52	<i>CYK3</i>	YDL117W		Role in cytokinesis	
53	<i>DFG5</i>	YMR238W	06073	Required for cell elongation, cell polarity	
54	<i>ELM1</i>	YKL048C	04755	Ser/Thr protein kinase in Swe1 pathway	
55	<i>END3</i>	YNL084C		Endocytosis and cytoskeletal organization	
56	<i>ENT1</i>	YDL161W	03598	Endocytosis and cytoskeletal organization	
57	<i>ENT2</i>	YLR206W	03598	Endocytosis and cytoskeletal organization	
58	<i>ENT3</i>	YJR125C	05992	Recruitment of clathrin	
59	<i>ENT4</i>	YLL038C	03598	Endocytosis	
60	<i>FLO8</i>	YER109C	01278	Required for pseudohyphal growth	
61	<i>GIC1</i>	YHR061C		Cdc42 effector. Contains CRIB domain	
62	<i>GIC2</i>	YDR309C		Cdc42 effector. Contains CRIB domain	
63	<i>GIN4</i>	YDR507C	03928	Protein kinase involved in bud growth. Similar to Kcc4 and Hsl1	
64	<i>HOF1/CYK2</i>	YMR032W	00168	Required for cytokinesis.	
65	<i>HSL1</i>	YKL101W	03928	Nim-related protein kinase; septin ring assembly; Swe1 pathway	
66	<i>HSL7</i>	YBR133C	15057	Involved in Swe1 pathway	
67	<i>HYM1</i>	YKL189W	10613	Cell morphogenesis	
68	<i>IOG1</i>	YPL242C	10730	Cytokinesis. Cell morphology.	
69	<i>KCC4</i>	YCL024W	03928	Nim-related kinase	
70	<i>KEL1</i>	YHR158C	15019	Cell fusion and cell morphology	
71	<i>KEL2</i>	YGR238C	15019	Cell fusion and cell morphology	
72	<i>MSB1</i>	YOR188W		Cell morphogenesis. Involved in regulation of Pkc1 and 1,3-beta glucan synthase	
73	<i>MSB2</i>	YGR014W	00480	Multicopy suppressor of cdc24 bud emergence defect	
74	<i>MSB3</i>	YNL293W	00935	GTPase activating protein for Sec4; localizes to sites of polarized growth	
75	<i>MSB4</i>	YOL112W	00935	GTPase activating protein for Sec4; localizes to sites of polarized growth	
76	<i>MUB1</i>	YMR100W	02587	Cell separation defect; homologue of <i>A. nidulans</i> SamB	
77	<i>PEA2</i>	YER149C		Polarisome component.	
78	<i>PKC1</i>	YBL105C	15023	Ser/Thr protein kinase. Cell wall integrity. Localizes to sites of polarized growth.	
79	<i>PLC1</i>	YPL268W	02982	Phospholipase C. Pseudohyphal growth	
80	<i>PXL1</i>	YKR090W	01663	Maintenance of polarized growth. Similarity to metazoan paxillin	
81	<i>RAS1</i>	YOR101W	00986	GTPase involved in eAMP signalling pathway.	
82	<i>RAS2</i>	YNL098C	01643	Homologue of mammalian Ras proto-oncogene	
83	<i>RAX1</i>	YOR301W	00956	Bipolar budding pattern landmark	

CELL POLARITY/CELL MORPHOGENESIS

	Sc gene	Systematic name	<i>U. maydis</i> MIPS#	E value	Function in <i>S. cerevisiae</i>
84	<i>RAX2</i>	YLR084C	05194	2.4e-31	Bipolar budding pattern landmark
85	<i>RDJ1</i>	YDL135C	05693	7.8e-33	Rho GDP dissociation inhibitor for Cdc42
86	<i>RGAI</i>	YOR127W	03864	8.3e-44	GAP for Cdc42
87	<i>RGAI</i>	YDR379W	03864	2.0e-39	GAP for Cdc42
88	<i>RHO1</i>	YPR165W	05734	2.7e-78	Rho subfamily. Regulator of Pkc1 and 1,3-beta glucan synthase
89	<i>RHO2</i>	YNL090W	02494	6.4e-54	Rho subfamily protein.
90	<i>RHO3</i>	YIL118W	04070	8.7e-52	Rho subfamily protein.
91	<i>RHO4</i>	YKR055W	10663	8.9e-48	Rho subfamily protein.
92	<i>RHO5</i>	YNL180C	00295	3.5e-52	Rho subfamily protein.
93	<i>ROM1</i>	YGR070W	15070	5.9e-104	GEF for Rho1
94	<i>ROM2</i>	YLR371W	15070	2.0e-137	GEF for Rho1
95	<i>RVS161</i>	YCR009C	05283	4.1e-66	Amphiphysin-like lipid raft protein. Regulates polarization of the actin cytoskeleton.
96	<i>RVS167</i>	YDR388W	01748	1.4e-51	Regulation of actin cytoskeleton
97	<i>SAC7</i>	YDR389W	02902	1.2e-43	GAP for Rho1 (Functionally related to Bag7)
98	<i>SHS1/SEP7</i>	YDL225W	03449	3.7e-72	Septin
99	<i>SPA2</i>	YLL021W	04468	5.6e-20	Polarisome component
100	<i>SPH1</i>	YLR313C	04468	2.3e-10	Polarisome component. Homologous to Spa2
101	<i>SWE1</i>	YIL187C	06337	5.3e-40	Ser/Thr protein kinase; involved in switch from polarized to isotropic growth
102	<i>WSC1/HCS77</i>	YOR008C	11546	3.1e-21	Stretch receptor: cell wall integrity pathway
103	<i>WSC2</i>	YNL283C		no hits	Stretch receptor: cell wall integrity pathway
104	<i>WSC3</i>	YOL105C	15084	1.6e-28	Stretch receptor: cell wall integrity pathway
105	<i>WHI3</i>	YNL197C	04835	2.3e-23	Regulator of cell size. RNA binding protein
106	<i>YCK1</i>	YHR135C	00274	3.4e-129	Casain kinase I isoform. Cell morphogenesis
107	<i>YCK2</i>	YNL154C	00274	1.6e-126	Casain kinase I isoform. Cell morphogenesis. Functionally redundant with Yck1.
108	<i>ZDS1</i>	YMR273C	15061.2	4.7e-20	Regulation of cell polarity
109	<i>ZDS2</i>	YML109W		no hits	Regulation of cell polarity. Paralogue of ZDS1

ACTIN CYTOSKELETON

	Sc gene	Systematic name	<i>U. maydis</i> MIPS#	E value	Function in <i>S. cerevisiae</i>
110	<i>ABP1</i>	YCR088W	05340	7.9e-23	Actin-binding protein
111	<i>ACE4</i>	YUR083C			Actin cytoskeleton organization
112	<i>ACT1</i>	YFL039C	11232	2.0e-185	Actin
113	<i>AIP1</i>	YMR092C	05949	3.0e-86	Actin-interacting protein; actin patch component
114	<i>APP1</i>	YNL094W	04613	9.7e-22	Actin filament organization
115	<i>ARC15</i>	YIL062C	02388	2.6e-25	Subunit of Arp2/3 complex
116	<i>ARC18</i>	YLR370C	10246	7.4e-44	Subunit of Arp2/3 complex
117	<i>ARC19</i>	YKL013C	10173	9.8e-58	Subunit of Arp2/3 complex
118	<i>ARC35</i>	YNR035C	11479	1.8e-49	Subunit of Arp2/3 complex
119	<i>ARC40</i>	YBR234C	05906	3.7e-69	Subunit of Arp2/3 complex
120	<i>ARK1</i>	YNL020C	03081	8.7e-61	Actin regulating Ser/Thr kinase
121	<i>ARP2</i>	YDL029W	05405	1.7e-149	Subunit of Arp2/3 complex
122	<i>ARP3</i>	YJR065C	11265	1.2e-139	Subunit of Arp2/3 complex
123	<i>BNJ1</i>	YNL271C	12254	1.9e-59	Formin; actin nucleation
124	<i>BNR1</i>	YIL159W	01141	2.3e-31	Formin; actin nucleation. Functionally redundant with Bni1
125	<i>BSP1</i>	YPR171W		no hits	Involved in actin cytoskeleton organization
126	<i>CAP1</i>	YKL007W	00423	1.1e-24	F-actin capping protein alpha subunit
127	<i>CAP2</i>	YIL034C	11177	2.0e-50	F-actin capping protein beta subunit
128	<i>CCT1/TCPI</i>	YDR212W	01279	3.4e-213	Chaperonin role for actin and tubulin
129	<i>CCT2</i>	YIL142W	06235	5.5e-190	Chaperonin role for actin and tubulin
130	<i>CCT3</i>	YIL014W	06067	1.1e-198	Chaperonin role for actin and tubulin
131	<i>CCT4</i>	YDL143W	02571	9.7e-122	Chaperonin role for actin and tubulin
132	<i>CCT5</i>	YJR064W	03959	4.6e-186	Chaperonin role for actin and tubulin

ACTIN CYTOSKELETON					
	Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
133	<i>CCT6</i>	YDR188W	02350	6.5e-167	Chaperonin role for actin and tubulin
134	<i>CCT7</i>	YIL111W	00565	8.2e-196	Chaperonin role for actin and tubulin
135	<i>CCT8</i>	YJL008C	04401	2.6e-151	Chaperonin role for actin and tubulin
136	<i>CHC1</i>	YGL206C	03921	0.0	Clathrin heavy chain
137	<i>COF1</i>	YLL050C	04314	3.1e-45	Actin binding and severing protein
138	<i>CRN1</i>	YLR429W	04417	6.5e-107	Cortical actin cytoskeleton component
139	<i>LAS1</i>	YKR063C	02271	6.9e-18	Bud formation and morphogenesis
140	<i>LAS17/BEE1</i>	YOR181W	03687	2.8e-51	Activator of the Arp2/3 complex. Homologue of human WASP
141	<i>MLC1</i>	YGL106W	11848	4.0e-29	Mvosin light chain
142	<i>MLC2</i>	YPR188C	03910	5.1e-13	Mvosin light chain
143	<i>MYO1</i>	YHR023W	03286	5.4e-183	Type II mvosin
144	<i>MYO2</i>	YOR326W	04555	2.4e-276	Type V mvosin
145	<i>MYO3</i>	YKL129C	11115	0.0	Type I mvosin
146	<i>MYO4</i>	YAL029C	04555	4.5e-258	Type V mvosin
147	<i>MYO5</i>	YMR109W	11115	0.0	Type I mvosin
148	<i>PAN1</i>	YIR006C	11804	1.3e-40	Actin patch protein
149	<i>PPY1</i>	YOR122C	10832	2.0e-27	Profilin
150	<i>PRK1</i>	YIL095W	03081	9.5e-60	Ser/Thr protein kinase; regulation of organization and function of actin
151	<i>SAC6</i>	YDR129C	04768	2.6e-218	Fimbrin; actin filament bundling protein
152	<i>SCP1</i>	YOR367W		no hits	Component of cortical actin cytoskeleton
153	<i>SLA1</i>	YBL007C	05337	8.3e-57	Assembly of cortical actin cytoskeleton
154	<i>SLA2</i>	YNL243W	00582	1.3e-78	Assembly of cortical actin cytoskeleton
155	<i>SRV2</i>	YNL138W	10957	1.4e-98	Regulation of actin dynamics and cell morphogenesis
156	<i>VRP1</i>	YLR337C	10566	3.7e-43	Verprolin. Regulation of actin cytoskeleton. Homologue of human WIP
EXOCTYST					
	Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
157	<i>SEC3</i>	YER008C	01107	7.2e-27	Spatial landmark for secretion
158	<i>SEC5</i>	YDR166C	00710	4.9e-22	Subunit of the exocyst complex
159	<i>SEC6</i>	YIL068C	03632	1.6e-67	Subunit of the exocyst complex
160	<i>SEC8</i>	YPR055W	02554	2.6e-35	Subunit of the exocyst complex
161	<i>SEC10</i>	YLR166C	00329	1.2e-61	Subunit of the exocyst complex
162	<i>SEC15</i>	YGL233W	05494	1.1e-29	Subunit of the exocyst complex
163	<i>EXO70</i>	YJL085W	01845	4.3e-14	Subunit of the exocyst complex
164	<i>EXO84</i>	YBR102C	04147	6.5e-31	Subunit of the exocyst complex
OTHER (SECRETION)					
	Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
165	<i>SEC4</i>	YFL005W	03865	7.2e-62	Rab GTPase. Associates with Sec15
166	<i>SEC2</i>	YNL272C	00553	7.3e-23	GEF for Sec4
MICROTUBULE CYTOSKELETON (SELECTED GENES) DYNEIN/DYNACTIN (per <i>S. cerevisiae</i>) [*]					
	Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
167	<i>DYN1/DHC1</i>	YKR054C	15045	0.0	Heavy chain of cytoplasmic dynein
168	<i>DYN2/SLC1</i>	YDR424C	04372	2.6e-150	
169	<i>DYN3</i>	YMR299C	12278	9.1e-23	Dynein light chain
170	<i>PAC11</i>	YDR488C	04598 ^{&}	no hits	Dynein intermediate chain. Nuclear migration
171	<i>ACT5/ARP1</i>	YHR129C	11692	4.2e-105	Component of the dynactin complex; related to centractin
172	<i>ARP10</i>	YDR106W	05833 ^{&}	no hits	Component of the dynactin complex
173	<i>JNM1</i>	YMR294W	02603 ^{&}	no hits	Component of the dynactin complex
174	<i>NIP100</i>	YPL174C	03826	6.6e-17	Component of the dynactin complex. Putative orthologue of mammalian p150 ^{Gluc}

<i>KINESINS (per S. cerevisiae)[#]</i>				
Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
175	YELO61C	10678	1.0e-96	Kinesin. Mitotic spindle assembly; chromosome seegregation. Related to Kip 1
176	YPR141C	11986	1.6e-80	Kinesin (- end directed) Related to Um Kin14
177	YBL063W	10678	2.5e-107	Kinesin. Functionally redundant with Cin8
178	YPL155C	00896	5.4e-52	Kinesin related motor protein Related to Um Kin7a and Kin7b
179	YGL216W	01560	1.7e-118	Kinesin related motor protein Related to Um Kin8
180	SMY1	04218	3.4e-45	Interacts with Mvo2. N-terminal domain related to motor domain of kinesins.
+TIPs				
Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
181	BIK1	06338	2.5e-13	MT plus end associated protein. Orthologue of mammalian CLIP170
182	BIM1/YEB1	05761	7.5e-51	Together with Kar9 makes up the MT capture site at the cell cortex
183	KAR9		no hits	Nuclear migration
184	PAC1	03164	7.8e-36	LIS1 /NudF homologue. Nuclear migration
TUBULIN				
Sc gene	Systematic name	<i>U. maydis</i> MIPS [#]	E value	Function in <i>S. cerevisiae</i>
185	TUB1	01221	4.4e-181	α -tubulin
186	TUB2	05828	6.7e-185	β -tubulin
		10558	2.0e-160	β -tubulin
187	TUB3	01221	1.5e-180	α -tubulin
188	TUB4	03803	1.1e-90	γ -tubulin

* Components of the *U. maydis* dynactin complex shown are per comparison with *S. cerevisiae*.
& Blast analysis indicates that there are no hits for these genes, however, the *U. maydis* gene numbers provided appears to correspond to the yeast gene.
[#] Kinesins shown are per comparison with *S. cerevisiae* kinesins. See article by Schuchardt et al., 2005 for details of the kinesin superfamily in *U. maydis*.