The published article appeared in: Trends Cell Biol. 2008 Feb;18(2):61-67.

Ustilago maydis, a new fungal model system for cell biology

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Running title: The model *Ustilago maydis*
Abbreviations: MT, microtubule; MTOC, microtubule organizing center

Abstract

Studies using fungi are closely related to animals. Fungal model systems, such as *Saccharomyces cerevisisae* and *Schizosaccaromyces pombe*, have contributed enormously to our understanding of essential cellular processes. Here we introduce the corn smut fungus *Ustilago maydis* as a new model organism to study cell biological processes. Genome wide analysis demonstrates that *U. maydis* is more closely related to humans than to budding yeast, with numerous proteins being shared only by *U. maydis* and *Homo sapiens*. Growing evidence suggests that basic principles of long-distance transport, mitosis or motor-based microtubule organization are conserved between *U. maydis* and humans. The fungus *U. maydis* therefore offers a unique system for the study of certain mammalian processes.

Introduction: Fungal model systems in cell biology

Fungi are simple eukaryotes that are surrounded by a cell wall and that grow by polar tip growth. Consequently, fungi were traditionally considered to belong to the kingdom of plants and it was not until the late 1960s that fungi were considered to be a new kingdom [1]. Nowadays, comparative analyses of rRNA and protein sequences have shown that fungi are even more closely related to animal cells than previously thought [2, 3]. This evolutionary relationship combined with the amenability of fungi to powerful techniques, such as classical and molecular genetics, has established fungi as powerful model systems for fundamental cell biological questions. The ascomycete yeasts *Saccharomyces cerevisiae*, and *Schizosaccaromyces pombe* have provided unique insights into the molecular basis of cell cycle control [4] and the molecular basis of chromatin remodelling during transcription [5]. In addition, genetic experiments in filamentous fungi, such as the ascomycete *Aspergillus nidulans*, have led to the important discoveries, including the identification of the tubulin genes [6] or mitotic kinesins [7]. However, some essential processes, such as nuclear envelope dynamics during open mitosis in animal cells are not found in ascomycete model fungi. The study of such processes would therefore benefit greatly from an equivalent model system. In this review we describe the dimorphic fungus *Ustilago maydis*. This basidiomycete is long known to be the

cause of corn smut disease in *Zea mays* (Box 1). Recent advances by numerous laboratories have established a broad range of molecular, genetic, bioinformatic and cell biological techniques (Table 1), and together with the public genome data [8] have made *U. maydis* a well-recognized model system in molecular plant pathology [9, 10]. However, recent advances in our understanding of the basic cell biological processes in *U. maydis* have suggested that this fungus is in many respects closely related to metazoans than to its ascomycete cousins, and could be an effective tool for the investigation of some cellular processes. In this review we describe the recent progress in *U. maydis* research, and propose this organism as a model system to elucidate basic cell biological process in higher eukaryotes such as long-distance transport, mitosis or motor-based microtubule organization.

DNA-repair: insights from *U. maydis*

Pioneering work in the 1960s by Robin Holliday in *U. maydis* demonstrated for the first time that DNA repair and recombination share enzymatic steps. Moreover, the mechanism of homologous recombination, involving the “Holliday junction” was initially proposed based on work in this fungus (for review see Ref. [11]). In the following years, the yeast *S. cerevisiae* became a more common model system for analyzing DNA repair, and much of what we know today about these processes comes from studies in this yeast [12]. However as the *S. cerevisiae* lacks some of the important components involved in DNA repair in animal cells, it limits the use of this fungal model system for understanding the molecular basis of tumorgenesis in mammalians and hence much investigation into cancer mechanisms is undertaken in mammalian cell lines. One of these components is the breast cancer susceptibility protein BRCA2. Defects in this large protein cause inherited receptiveness to breast, ovarian and other cancers [13]. BRCA2 homologues are not found in budding yeast or another genetically tractable system, *Drosophila melanogaster* [14]. However, during a genetic screen of DNA repair-defective mutants of *U. maydis*, a *BRCA2* homologue, *Brh2*, was discovered [15], opening up a new avenue for BRCA2 research. Although the Brh2 is only approximately a third of the size of vertebrate BRCA2 protein, all BRCA2

ascribed functions in DNA repair were present in *U. maydis* Brh2. Moreover, the smaller size of Brh2 allowed the characterization of its activities *in vitro*. This led to the discovery that Brh2 recruits Rad51 to DNA and facilitates the nucleation of the recombinogenic complex [16], which provided experimental support to a long- existing hypothesis. Thus, research on DNA repair and recombination in *U. maydis* not only has a long history back to the pioneering work of Robin Holliday, it still provides novel insights into this essential process in metazoans.

U. maydis and neurons share phenotypic similarities

Unlike yeasts, filamentous fungi, such as *A. nidulans* and *U. maydis* form elongated hyphal cells that expand by polar tip growth. In this respect fungal hyphae are phentypically similar to mammalian neurons that also progress their elongated axon by expansion of the apical growth region. It was in particular the work in *A. nidulans* by R. Morris, X. Xiang and co-workers that demonstrated the value of filamentous fungi as models for neurons, which is best demonstrated by their finding that defects in dynein-based transport most likely underlies human neuro-degenerative Lissencephaly [17]. In neurons and in *U. maydis* microtubules, which are dynamic polymers of tubulin, form long arrays that serve as "tracks" for directed intracellular long-distance transport along the hyphae or axon. The process of long- distance transport along microtubules is crucial in brain development and defects in axonal transport cause numerous severe neurodegenerative diseases [18]. In neurons transport towards the axonal growth cone (anterograde) is mediated by kinesins, whereas dynein supports retrograde traffic from the synapses to the cell soma [19]. Anterograde microtubule-based traffic of membranes is mainly based on motors belonging to the kinesin-1 and kinesin-3 family [20]. Interestingly, in *U. maydis* hyphal growth also requires kinesin-1 and kinesin-3 motors. So far the membranous cargo of kinesin-1 in *U. maydis* is elusive, kinesin-3 has been shown to counteract dynein in rapid bi-directional transport of early endosomes [21], a process that also involves kinesin-3 motors in animal cells [22]. Another intriguing similarity is the observation that kinesin-1 and myosin-V colocalize and cooperate in order to allow polarized cell extension in *U. maydis* [23]. a functional relationship that was initially suggested for animal cells [24]. Surprisingly, kinesin-3 is not found

in *S. pombe,* and *S. cerevisiae* even lacks both kinesin-1 and kinesin-3. This suggests that the basic machinery for long-distance transport is an ancient inventory of fungi and animals, which got lost during the evolution of the small yeasts.

In addition to transport of proteins and membranes, neuronal growth requires microtubule-based long-distance transport of RNA, whereas short distances are bridged by actin and myosin motors [25]. A short distance transport process is also required in *S. cerevisiae*, where actin cables and myosin-V motors mediates RNA traffic [26]. However, studies on putative RNA-binding proteins in *U. maydis* have strongly suggested that microtubules are required for long-distance transport of RNA in fungal hyphae [27], again suggesting that the phenotypic similarity of fungal hyphae and axons require similar transport mechanisms. Thus it appears that studies in both *S. cerevisiae* and *U. maydis* together can recapitulate the mammalian system.

In neurons, microtubules are formed at nucleus-associated microtubule-organizing centres, but are subsequently released and are taken into the axon and the dendrites by the activity of molecular motors [28]. A major player in the motor-driven organization of the neuronal microtubule array is cytoplasmic dynein that is thought to push microtubules with their plus-ends leading into the axon [29]. For a long time such dynein-based transport and organization of interphase microtubules was only known in animal cells. However, work in *U. maydis* has recently demonstrated that this process is evolutionarily ancient. Live cell observation of green fluorescent protein-labelled microtubules showed that assembled tubulin polymers move throughout the *U. maydis* cell at rates typical for motor activity [30]. Like in mammalians microtubules are transported with their plus-end leading, and this process depends on the activity of dynein [31]. Interestingly, without functional dynein the polarity of the microtubule array in *U. maydis* is lost. Thus, it appears that in *U. maydis* and in neurons dynein-based transport of microtubules organizes and polarizes the microtubule array. Interestingly, kinesin-1 also participates in microtubule organization in *U. maydis* [32]. This is mediated by microtubule-microtubule cross-linking, which involves a second microtubule-binding region at the conserved tail of the motor,

suggesting that fungal kinesin-1 is able to move two microtubules relative to each other. Results from *in vitro* experiments indicate a similar function might exist in animal kinesin-1 [33, 34], but whether this motor participates in microtubule organization in living animal cells remains to be shown.

Cell polarity is controlled by both intra- and extracellular signals, which must be integrated to give the final shape of the cell. In neurons, Cdk5, a cyclin-dependent kinases with morphogenic functions [35], is an important master regulator of different aspects of neuronal polarity [36]. Among the different targets of neuronal Cdk5 are small GTPases such as Rac1 [37]. All fungi contain Cdc42 and Rho-like GTPases, but Rac1 is restricted to filamentous fungi. In *U. maydis* Rac1 controls polarized hyphal growth [38] Interestingly, a Cdk5-like kinase also plays an essential role in sustained polar growth in *U. maydis* and, similarity to neuronal Cdk5, the *U. maydis* counterpart regulates the activity of Rac1 [39]. Thus, it is intriguing to speculate that neurons and hyphae of *U. maydis* not only share the basic inventory for long-distance transport machinery, but they also share similarities at the level of the control of polarized growth.

Metazoan-like mitosis in *U. maydis*

Interphase chromosomes are surrounded by the nuclear envelope, which contains the nuclear pores that mediate communication between the interior of the nucleus and the cytoplasm. In higher eukaryotes, the nuclear envelope breaks down, the nuclear pores disassemble at the onset of mitosis and a cytoplasmic spindle is formed (“open mitosis”; [40]). In contrast, in most fungal systems the mitotic nuclear envelope and the nuclear pore complex remains intact and the spindle forms intranuclear. This "closed" mitosis is characteristic for ascomycete fungi [41], although recent results in *A. nidulans* demonstrate that nuclear pores partially disassemble [42]. Early ultrastructural data [43] and recent live cell imaging [44] demonstrated that *U. maydis* removes the mitotic nuclear envelope. At the onset of mitosis the nucleus elongates and the envelope opens at the tip. Subsequently, the chromosomes leave the nuclear envelope, which collapses and is recycled in later stages of mitosis. Whereas

this is in contrast to mammalians, where the envelope disassembled in prophase, dynein is required for nuclear envelope break down in both animals [45] and *U. maydis* [44]. In both systems the nuclear pores also disassemble during open mitosis [46, 47] and nucleoporins of the Nup107-160 complex are recruited to the condensing chromosomes in metaphase and early anaphase. However, whereas animal Nup107 localizes to kinetochores [48], the fungal counterpart showed no overlap with kinetochore markers but was exclusively found on the chromosomes themselves [47]. Later in anaphase proteins of the Nup107-160 complex are the first nucleoporins that reappear at the rim of the separating chromosome masses. This suggests that it might have a role in reassembly of the nuclear pores, which was shown for animals [49]. Thus, the disassembly/assembly cycle of the nuclear pores in *U. maydis*, and in particular the dynamic behaviour of Nup107 is reminiscent of animal cells. Considering the complexity of the process it therefore appears likely that the “open mitosis” is an ancient process that in part conserved between animals and fungi, but was given up in evolution of the ascomycete fungi.

During the "open" mitosis of metazoan cells, the spindle apparatus forms in the cytoplasm. Chromosomes move to the spindle poles in anaphase A and are segregated by rapid spindle elongation in anaphase B. In *Drosophila melanogaster* anaphase B is supported by cytoplasmic dynein that is thought to exert pulling forces on astral microtubules thereby positioning and elongating the spindle [50]. In contrast, spindle elongation in anaphase in the budding and the fission yeast is driven by kinesin-5 [51, 52], and dynein-based pulling forces appear not to participate in spindle elongation [53, 54]. In *U. maydis* spindle elongation starts with a slow initial phase (called anaphase A) that also requires kinesin-5 [55]. However, in contrast to the yeasts this is followed by a rapid second phase (anaphase B) that separates the chromosomes over distances of up to 20 µM. This rapid movement depends on dynein, which localizes to the tips of astral microtubules from where it exerts pulling forces on the spindle [55]. Thus, the mechanistic of mitotic spindle elongation is surprisingly similar between *U. maydis* and animal cells. The reason why *U. maydis* differs from the yeasts is not known, but it might be related to the larger cell dimensions of *U.*

maydis that require a rapid mechanism for chromosome segregation in order to keep mitosis short.

An unexpected high degree of similarity between humans and *U. maydis* mitosis was also found on the regulatory level of cyclins and cyclin-dependent kinases. In human cells, cyclin A is thought that the levels of cyclin A-CDK2 activity is a rate-limiting component for entry from G2 into mitosis and there is a strict correlation between cyclin levels and time spent in G2 phase [56]. A similar situation has been reported for the cyclin Clb2 in *U. maydis* [57]. It is worth noting that in neither *S. pombe* nor in *S. cerevisiae* are levels of mitotic cyclins rate-limiting for G2/M transition and therefore overexpression of these cyclins do not accelerate the entry into mitosis [64]. The reason for the difference between *U. maydis* and the yeasts is not known. However, it is possible that redundant controls for mitotic transitions exist in *S. pombe* and in *S. cerevisiae*, as both yeasts contain numerous mitotic cyclins, whereas *U. maydis* only has one.

In vertebrates the Polo kinase Plk promotes G2/M transition by co-ordinately controlling both positive and negative regulators of Cdc2 [58]. In *U. maydis* Polo kinases might perform similar roles, as conditional mutants arrest in G2-phase (Mielnichuk and Perez-Martin, unpublished result). These similarities on the level of cell cycle regulation raise the possibility that work on *U. maydis* could lead to the identification of new mitotic regulatory loops in metazoans. One recent example might be the role of the mitotic exit network (MEN) in mitosis. In the yeasts *S. cerevisae* and *S. pombe* this pathway is involved in the termination of mitosis and in subsequent cytokinesis and septation [59]. However, in *U. maydis* the MEN-related signalling network participates in the nuclear envelope breakdown in prophase [44, 60]. Elements of this signalling network are also present in humans [61], and it will be exciting to ascertain whether MEN has a role in nuclear envelope breakdown in mammalian cells, or if it functions more akin to its role in yeast.

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A world to be discovered: What the *U. maydis* genome offers to metazoan research

It was mentioned above that *U. maydis* and mammalians share proteins that are not found in *S. cerevisiae* and *S. pombe* (e.g. kinesin-3). Recently, a genome-wide comparison of the predicted proteome of *U. maydis*, *S. cerevisiae* and humans was undertaken [62]. The surprising outcome was that *U. maydis* shares more protein sequence similarity with humans than with its fungal cousin *S. cerevisiae* (Figure 2; left panel show the result of an analysis at a simple cut-off of 20% identity over the entire protein; right panel provides direct comparison of amino acid identity over the entire protein). When proteins were included that showed sequence similarity in conserved domains only the number of proteins shared by *U. maydis* and humans raised to 681 proteins, many of which where absent in the *S. cerevisiae* proteome (<10% identity). A functional prediction within this group of conserved proteins suggested that for certain functions, complete cellular processes might be conserved between *U. maydis*. On the other hand, humans and *S. cerevisiae* share 321 common proteins not existing in *U. maydis*. This nicely illustrates the value of a broader range of model systems, which might be used together in order to completely recapitulate human processes. Interestingly, among the proteins shown to be conserved between *U. maydis* and humans are many genes, including the homologues of the Benzodiazepine receptor BZRP and Carboxylesterase CES1, which are implied in cancer. However, most proteins shared by *U. maydis* and humans are without any functional prediction. Analysis of these proteins is required to build a complete picture of the conservation between *U. maydis* and the metazoan cell.

Conclusion

Molecular analyses have demonstrated that fungi are evolutionary related to animal cells. Consequently, the basic principles of many cellular processes are conserved between both groups. Fungal model systems, such as bakers and fission yeast have largely contributed to our understanding of essential cellular pathways. This is mainly due to their technical advantages, such as genetic accessibility, a short generation time, simple cultivation

methods and sophisticated molecular tools. The basidiomycete fungus *U. maydis* provide most of the technical advantages of the yeasts, and, similar to other filamentous fungi, the elongated hyphae have phenotypic similarities with neuronal cells. Functional analysis of some specific processes, such as the role of motors in MT organization, long-range transport, or the "open" mitosis in *U. maydis* revealed an unexpected degree of similarity between this fungus and higher eukaryotes. It is not known whether this will extend to other cellular processes, but the unexpected finding that the genome of *U. maydis* is closely related to humans further supports the utilization of *U. maydis* as a model system. Addressing the roles of the many proteins conserved in humans and *U. maydis* that are lacking any functional information remains a sizable challenge to those in the field, but one that once accomplished promises to expand our understanding of the basic biology of human cells.

Acknowledgement

We wish to thank Dr. Martin Münsterkötter for providing parts of figure 2, Juliane Klärig for generating strain AB33GT_nRFP that was used in figure 1, and Dr. Karen Brune for helpful comments on the manuscript. I apologize to those colleagues whose work could not be cited due to space constrictions. Our work is supported by the Deutsche Forschungsgemeinschaft (G.S.), the Max-Plank Institute for Terrestrial Microbiology in Marburg (G.S.) and Grants to J.P.M. from Spanish government (BIO2005-02998) and EU (MRTN-CT-2005-019277).

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Figure legends

Figure 1. Morphological stages of *U. maydis*.

A. In liquid culture haploid sporidia grow as yeast-like cells. They contain a single nucleus (A; red florescent protein was targeted to the nucleus) that is positioned in the middle of the mother cell, while the polar bud is formed. Upon mitosis, the nuclear envelope is removed and the nuclear proteins are released (arrow; the cell is in late anaphase). Microtubules are formed at cytoplasmic nucleation sites and reach into the bud (A; microtubules are visualized by green florescent protein fused to α-tubulin). B. Under natural conditions two mating partner cells recognize each other on the plant surface and fuse, which results in the assembly of the *b*-transcription factor and the formation of bi-nucleated hypha. In a mono-nucleated mutant strain that expresses both halves of the heterodimeric *b*-transcription factor under the control of inducible promoters (Table 1, strain AB33; [63]) filamentous growth can be induced by a medium

shift. Again microtubules span the entire length of the hyphae (MT, green) and the nucleus is positioned close to the middle of the cell (Nuclei, red) Bar: 10 µm.

Figure 2. Genome wide comparison of *U. maydis*, *S. cerevisiae* and *H. sapiens*. **A**. Sequence comparison of predicted proteins in *U. maydis*, *S. cerevisiae* and *H. sapiens* demonstrate that both fungi share a surprisingly low number of fungal specific proteins that are not found in the human genome (yellow; listed are proteins with >20 identity over the entire protein length). *U. maydis* shares a large number of genes (777) that are >20% identical to human, but less than 20% identical to bakers yeast. However, humans and *S. cerevisiae* also have a set of 514 in common. 1738 proteins show >20% sequence identity in all three organisms. Number of predicted genes in each organism is given in parenthesis. Note that genomes were corrected for gene duplications. Figure modified from [62].
B. Scatter plot showing a detailed comparison of proteins from *U. maydis* with *S. cerevisiae* and *H. sapiens* that is based on a more stringent criterion (20% amino acid sequence identity with one organism and more than 10% less amino acid sequence identity with the counterpart in the other organism; cut off is indicated by blue lines). Again, *U. maydis* shares more proteins with *H. sapiens* than with *S. cerevisiae*. Note the same criteria lead to 312 proteins that are shared bakers yeast and humans, but that are less conserved or not found in *U. maydis* [62].

Box1_Figure 1. Pathogenic development of *U. maydis*.
Yeast-like sporidia of *U. maydis* obtain their nutrients from dead organic matter (saprotrophic) and grow by polar budding. When in contact with the surface of the plant the pathogenic program is initiated by the exchange of pheromone (Box Fig. 1,“Pheromone exchange”) and a switch to filamentous growth (Box Fig. 1, “Dimorphic switch”). This results in long conjugation hyphae that fuse and form a hypha that contains two nuclei (dikaryotic; Box Fig. 1,“Cell fusion”). This filament enters the plant (Box Fig. 1,“Plant invasion”) and, after a short period of spreading within the host, starts to induce plant tumors, within which the fungus proliferates. After nuclear fusion (Box Fig. 1,“Nuclear fusion”) and fragmentation of the hyphae diploid black thick-walled resting spores

(teliospores) are formed (Box Fig. 1,“Spore formation”). These spores are release and form a promycelium that undergoes meiosis (Box Fig. 1,“meiosis”) and forms haploid sporida. Figure modified from [64].

Box 1. Pathogenicity of *U. maydis*

In the late 19th century botanists discovered the basidiomycota fungus *Ustilago maydis* as the causal agent of corn smut disease in maize. Due to its technical advantages (see Table1), *U. maydis* became a well-established model system in molecular plant pathology research (see [9, 10]). An *U. maydis* infection is characterized by the fungus-induced formation of so-called tumors in stem, leaves and flowers. Within the tumors the fungus proliferates and forms the dark diploid spores. After release these spores rest until they find conditions for germination and promycelium formation. After meiosis, the multi-cellular promycelium generates haploid yeast-like cells (sporidia; Box Figure 1) that live on dead organic matter and colonize the soil, where they proliferate by polar budding. Pathogenic development begins on the surface of young plants. Two compatible sporidia recognize each other by a pheromone-receptor system that is encoded by the *a*-mating locus [65]. Pheromone perception induces a cascade of regulatory events and leads to a cell cycle arrest in G2-phase [66], increased expression of pheromone receptor and pheromone and a switch from budding to hyphal growth [9]. As a consequence, the cells form long conjugation hyphae (*a*-dependent hyphae). In their attempt to reach the mating partner each hypha can grow to a length of >100 µm [67], thereby bridging significant distances. Successful fusion of two compatible hyphae results in the formation of a heterodimeric transcription factor, encoded by the *b*-mating loci of both partner cells [68]. The *b*-transcription factor is a master regulator of the subsequent pathogenic development, and it establishes a stable *b*-dependent hypha. This consists of a long tip cell that, while growing over the epidermis, leaves vacuolated sections behind. Eventually the *b*-dependent hypha redirects its growth and invades the plant tissue. Within the host, the cell cycle arrest is released and hyphae colonize the plant tissue. This finally leads to the formation of plant tumors, within which the fungus proliferates and, after nuclear fusion and hyphal fragmentation, forms the diploid spores. Nowadays the intensive use of fungicides efficiently controls *U. maydis* infections and usually

keeps the grain yield loss below 5%. However, depending on climate conditions even these days this number can rise up to >50%, which demonstrates that this fungal pathogen has the potential to be a threat to men and is therefore listed as a potential bio-weapon by the Ad Hoc Group of the Biological Weapons Convention of the United States of America [69].

Tables and Figures

Table 1. Molecular tools for *U. maydis*

Tool	Description	reference
1. strains		
FB1, FB2, FB6a, FB6b	Haploid strains that differ in their mating loci *a1*, *a2*, *b1*, *b2*	Ref. [70]
SG200	Haploid strain, in which the *a1* allele was replaced by a composite *a* allele containing *mfa1*, *pra1*, and *mfa2*; due to this genotype the strain is solo-pathogenic and does not need to fuse with a mating partner	Ref. [71]
AB33	Haploid strain that carries two compatible *b*-alleles under the control of the inducible *nar1*-promoter (see below); shift to inductive medium triggers filamentous growth.	Ref. [63]
AB31	Haploid strain that carries two compatible *b*-alleles under the control of the inducible *crg1*-promoter (see below); shift to inductive medium triggers filamentous growth.	Ref. [63]
FBD11	Diploid strain that is solopathogenic and is often used to check lethality of genes.	Ref. [70]
2. Promoters		
Ptef	Constitutively active promoter that controls transcription of the gene for the translation elongation factor	Ref. [72]
Potef	Modified *tef* promoter in which two direct repeats of the synthetic fragment containing seven tetracycline – responsive elements	Ref. [72]
mfa	Regulates the expression of pheromone gene. It has a low basal activity, is strongly induced after pheromone stimulation	Ref. [73]
Pcrg1	Carbon-regulated promoter. Repressed when cells are growing in glucose as carbon source and induced when cells are using arabinose as carbon source.	Ref. [74]
Pnar1	Nitrogen-regulated promoter. Repressed when cells are growing with amonium as nitrogen source and induced when cells are using nitrate as nitrogen source.	Ref. [63]
3. Tet-system	Tetracycline-regulated system for gene expression	Ref. [75]
4. Sfi-integration system	Method which allows one to generate constructs for gene replacement without the need of cloning.	Ref. [76]
5. Genomic libraries	There are several genomic libraries constructed in self-replicating plasmids suitable for cloning by complementation	Ref. [77]

6. Self-replicating UARS plasmids	Set of plasmids that carry various antibiotic resistence and that replicate autonomously in *U. maydis*	Ref. [78]
7. Fluorescent proteins	Various fluorescent proteins have been used in *U. maydis*, including variants of the green fluorescent protein and monomeric red fluorescent protein and derivatives	Refs. [21, 44]
9. Mating assays	Cell -cell fusion and filamentous growth can be induced by growing compatible strains on solid charcoal medium	Ref. [70]
10. Synthetic pheromone	Short peptide that, when applied in liquid culture, induces the dimorphic switch in compatible yeast-like cells	Ref. [79]
11. Array technique	Custom Affymetrix arrays that cover ~90% of the predicted 6902 *U. maydis* genes	Ref. [8]
12. Bioinformatics	The MIPS *Ustilago maydis* Genome Database provides profound information on the predicted proteins (http://mips.gsf.de/genre/proj/ustilago/). This information is based on the high quality draft genomic sequence of the Broad Institute.	

Table 2. Processes in *U. maydis* and humans that are not found in the yeast model systems

1. DNA repair	
- the breast cancer susceptibility protein BRCA2 participates in DNA repair	Ref. [15]
2. Microtubule organization	
- Dynein transports assembled microtubules and polarizes the tubulin array	Ref. [31]
- Microtubules grow slowly but depolymerize rapidly	Ref. [30]
3. Long-distance transport	
- Kinesin-1 and myosin-5 cooperate in order to deliver material to the tip	Ref. [23]
- Kinesin-3 mediates traffic of early endosomes	Refs. [21, 80]
- Microtubules support transport of RNA-binding proteins	Ref. [27]
4. Polarized growth	
- Cdk5 controls polarity, most likely by affecting Rac1	Ref. [39]
5. Mitosis	
- Dynein removes the nuclear envelope in prophase	Ref. [44]
- Dynein supports spindle elongation in anaphase B	Ref. [55]
- The nuclear pores disassemble and Nup107-complex components are recruited to the chromosomes	Ref. [47]
- Cyclin A regulates G2/M transition	Ref. [57]
6. Predicted proteins	
- numerous proteins shared between *U. maydis* and human that are mainly without predicted function	Ref. [62]

Figure 1
Steinberg and Perez-Martin

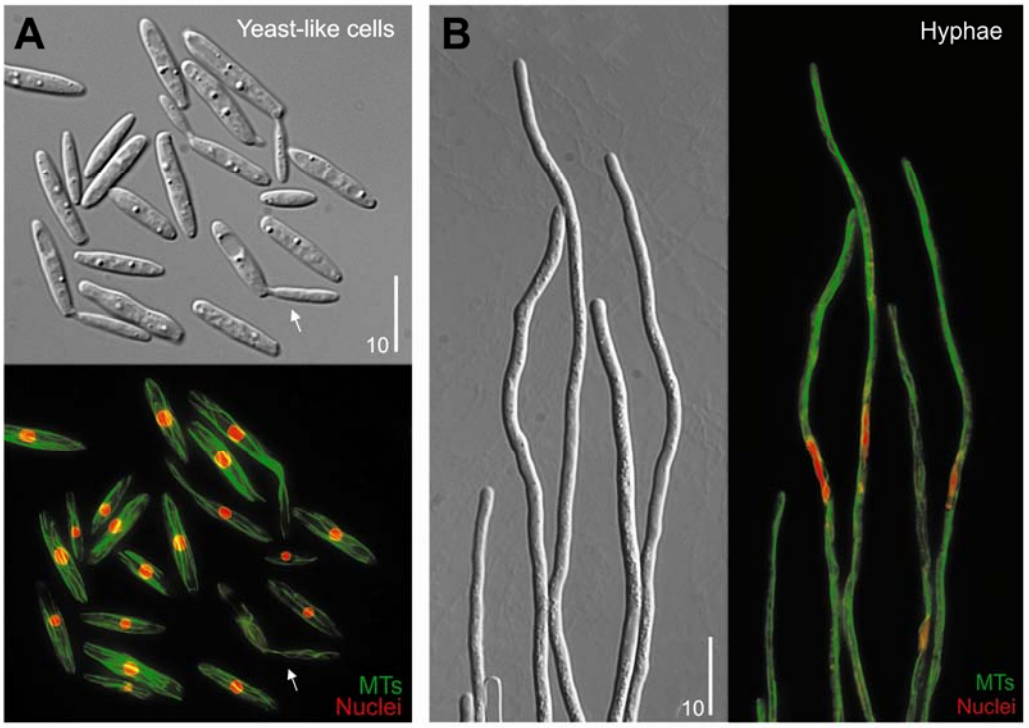
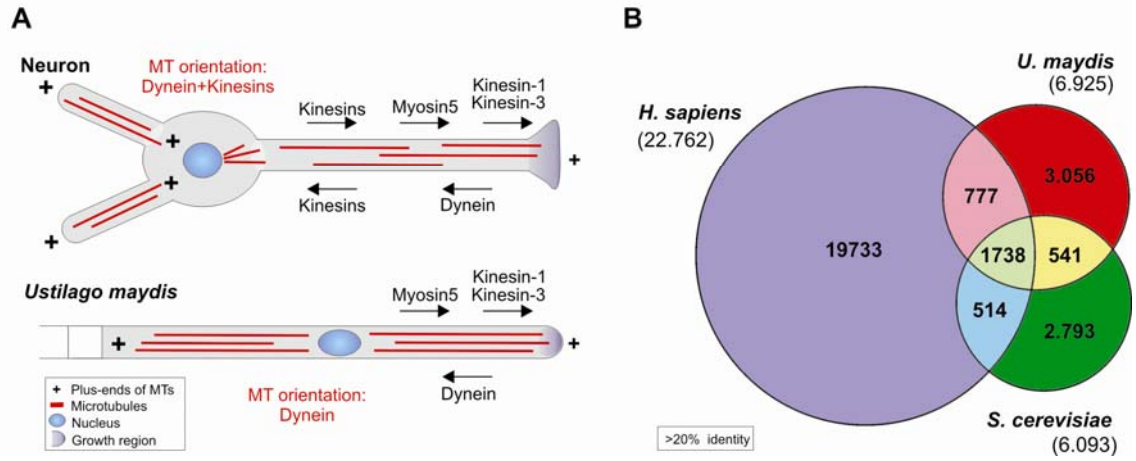
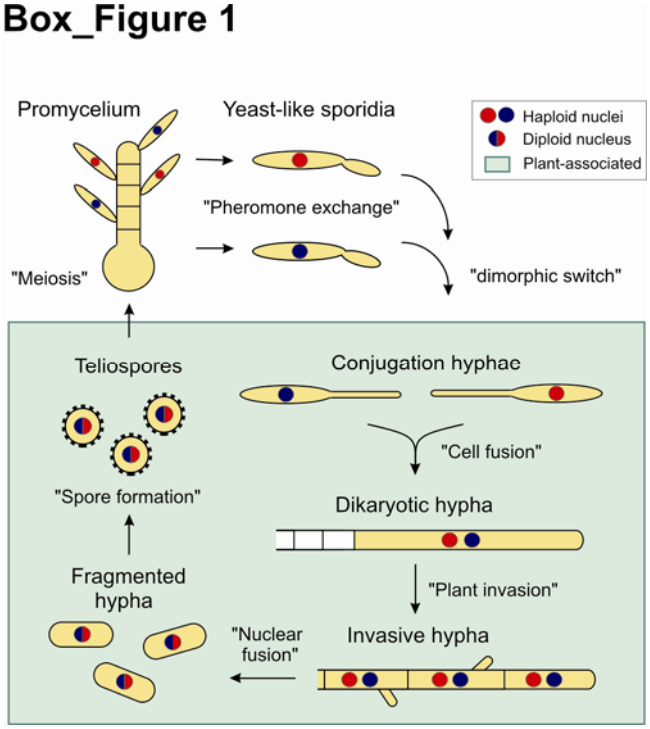


Figure 2
Steinberg and Perez-Martin



Box_Figure 1


Promycelium
Yeast-like sporidia
Haploid nuclei
Diploid nucleus
Plant-associated
"Pheromone exchange"
"Meiosis"
"dimorphic switch"
Teliospores
Conjugation hyphae
"Cell fusion"
"Spore formation"
Dikaryotic hyphae
"Plant invasion"
Fragmented hypha
"Nuclear fusion"
Invasive hypha