Fungal development of the plant pathogen *Ustilago maydis*

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**Abstract**

The maize pathogen *Ustilago maydis* has to undergo various morphological transitions for the completion of its sexual life cycle. For example, haploid cells respond to pheromone by forming conjugation tubes that fuse at their tips. The resulting dikaryon grows filamentously, expanding rapidly at the apex and inserting retraction septa at the basal pole. In this review, we present progress on the underlying mechanisms regulating such defined developmental programmes. The key findings of the postgenomic era are as follows: (1) endosomes function not only during receptor recycling, but also as multifunctional transport platforms; (2) a new transcriptional master regulator for pathogenicity is part of an intricate transcriptional network; (3) determinants for uniparental mitochondrial inheritance are encoded at the *α2* mating-type locus; (4) microtubule-dependent mRNA transport is important in determining the axis of polarity; and (5) a battery of fungal effectors encoded in gene clusters is crucial for plant infection. Importantly, most processes are tightly controlled at the transcriptional, post-transcriptional and post-translational levels, resulting in a complex regulatory network. This intricate system is crucial for the timing of the correct order of developmental phases. Thus, new insights from all layers of regulation have substantially advanced our understanding of fungal development.

**Introduction**

*Ustilago maydis* is a basidiomycete that infects corn (Fig. 1a) and serves as an excellent model for plant pathogenicity (Banuett, 1995; Nadal et al., 2008; Brefort et al., 2009). However, it is also known to be a delicacy in Central America for hundreds of years and has even found its place in the food industry (Fig. 1b–d). Extensive research over the last couple of decades established this microorganism as a model for a number of important cellular processes such as signalling, transcriptional and post-transcriptional regulation, molecular transport, cell cycle regulation, as well as DNA recombination and repair (Bölker, 2001; Kahmann & Kämper, 2004; Perez-Martin et al., 2006; Feldbrügge et al., 2008; Steinberg & Perez-Martin, 2008; Brefort et al., 2009).

The research on DNA recombination and repair is a superb example documenting how the mechanistic insights gained in *U. maydis* promote research into human health (Llorente & Modesti, 2009). The foundation for work on DNA recombination was laid by R. Holliday more than 40 years ago. He introduced the concept of the now famous Holliday junction of DNA recombination while studying *U. maydis* (Holliday, 1964, 2004).

More recent research on DNA repair identified the key factor Brh2, a BRCA2 family protein (Kojic et al., 2002, 2011). Its founding member is a human tumour suppressor encoded by a predisposition gene of hereditary breast cancer. Brh2 from *U. maydis* catalyses the assembly of active recombinase complexes (Yang et al., 2005) and acts in double-strand repair to reunite broken ends (Mazloum & Holloman, 2009b). In addition, it might even be involved in unconventional strand invasion during the repair of defective replication forks (Mazloum & Holloman, 2009a). Based on these studies, mammalian BRCA2 research has gained tremendous momentum in resolving its molecular function. The protein is now considered a universal recombinase regulator that is conserved from lower to higher eukaryotes (Thorslund & West, 2007; Thorslund et al., 2010).

One of the major breakthroughs for the *U. maydis* research community was the public release of the genome
sequence combined with a profound manual annotation that resulted in high-quality data currently curated in the database MUMDB at MIPS (Kämper et al., 2006; MIPS U. maydis database; and the Munich information centre for protein sequences, respectively; http://mips.helmholtz-muenchen.de/genre/proj/ustilago/). This spurred the application of new approaches such as transcriptome-wide DNA microarrays (Scherer et al., 2006; Zarnack et al., 2008; Heimel et al., 2010b), proteome-wide approaches (Böhmer et al., 2007), gene replacement strategies for efficient knockouts or more sophisticated promoter and gene fusions (Brachmann et al., 2004; Kämper, 2004; Garcia-Pedrajas et al., 2008). Establishing fluorescence proteins such as eGfp, mRfp, mCherry, Yfp, Cfp, photoactivatable Gfp and split-Yfp facilitated comprehensive in vivo localization, colocalization and interaction studies (Steinberg & Perez-Martín, 2008; Heimel et al., 2010a; Schuster et al., 2011b).

In this review, we will use the sexual life cycle of *U. maydis* as a blue print to describe recent findings focusing on the postgenomic era. Complementary information can be found in earlier reviews (Kahmann & Kämper, 2004; Feldbrügge et al., 2006) and in the special issue of *Fungal Genetics & Biology* dedicated to *U. maydis* (Kronstad, 2008).

For the sake of clarity, the life cycle is divided into four distinct phases: proliferation of haploid cells, mating, filamentation and plant infection. However, the results described here indicate that key players of the underlying cellular events are often important at several additional stages during the life cycle.

**Synopsis of the plant-dependent life cycle**

The saprophytic phase can be considered as the default state and starts with meiosis during the germination of diploid teliospores. The resulting haploid cells proliferate by budding (Fig. 1e–g). A prerequisite for infection is the mating of two compatible haploid cells, preferentially on the plant surface (Fig. 2). They recognize each other using a pheromone receptor system that consists of seven-transmembrane domain receptors and small lipopeptide pheromones (*pra1* and *mfa1*, respectively) encoded at the biallelic *a* mating-type locus (Fig. 3; Böcker et al., 1992; Spellig et al., 1994; Szabó et al., 2002). Active pheromone signalling results in the formation of conjugation tubes, which orient their growth along the pheromone gradient of the mating partner. Thereby, compatible partners approach each other.
and fuse at their tips, initiating plasmogamy (Fig. 2; Snetselaar et al., 1996). Characteristic for basidiomycetes, plasmogamy and karyogamy are separated in time (Kruzel & Hull, 2010). A stable dikaryon is formed that grows filamentously with a defined axis of polarity. Hyphae expand at the apical growth cone (Fig. 2) and insert retraction septa at regular intervals at the basal pole. These septa separate the viable compartment from hyphal segments devoid of a cytoplasm, resulting in the formation of regularly spaced empty sections (Lehmler et al., 1997; Steinberg et al., 1998). The nuclei travel to the centre of the cell and are positioned in a defined distance of about 10 μm from each other as well as about 50 μm from the tip (Steinberg et al., 1998; Fuchs et al., 2005).

The developmental switch resulting in hyphal growth is genetically controlled by the action of homeodomain transcription factors encoded as two separate subunits, bWest (bW) and bEast (bE), at the multiallelic b mating-type locus (Kronstad & Leong, 1990; Gillissen et al., 1992). The activity of the transcription factor is elegantly coupled to plasmogamy because it can only function as a heterodimer with subunits derived from different mating partners (e.g. bW1/bE19, bW5/bE8, etc.; Kämper et al., 1995). At present, 19 different b alleles that promote outbreeding are known (J. Kämper, pers. commun.; Barnes et al., 2004).

The dikaryotic filament is the infectious form of the fungus. It grows in close contact with the plant and is able to sense surface signals that trigger the formation of appressoria (Mendoza-Mendoza et al., 2009a; Lanver et al., 2010). These are specialized infection structures that enable the pathogen to enter the plant. Initially, hyphae grow intracellularly by invagination of the plant plasma membrane, establishing a tight interaction zone with colonized host cells (Snetselaar & Mims, 1993; Doehlemann et al., 2008b). At later stages, proliferation also occurs intercellularly and the dikaryotic mycelium grows towards bundle sheets (Doehlemann et al., 2008b). Subsequently, massive proliferation and hyphal fragmentation occurs. This is accompanied by an irregular division of host cells, resulting in the formation of tumours at all aerial parts of the plants. After karyogamy, black diploid teliospores develop within the tumours. Ripe tumours rupture and mature spores are spread by the wind. Under favourable conditions, teliospores germinate and release haploid cells. The life cycle begins anew.

**Proliferation of haploid cells and cytokinesis**

Saprophytic cells exhibit a defined cylindrical cell shape comparable to *Schizosaccharomyces pombe*. However, they do not divide by insertion of a central septum, but proliferate by budding like *Saccharomyces cerevisiae* (Fig. 1e–g). During proliferation, the bud expands by polar tip growth, presumably by actin-mediated secretion of remodelling enzymes and building blocks of the cell wall (Banuett & Herskowitz, 2002; Weber et al., 2006). This notion is supported by the observation that an actin-dependent class V myosin localizes to regions of polar growth and is important for cell morphology (Weber et al., 2003). After the daughter cell reaches a certain size, the nucleus migrates from the centre of the mother into the bud where division occurs (Holliday, 1974; O’Donnell & McLaughlin, 1984). At the mother/bud neck region, the nuclear envelope is striped off, nuclear pore complexes are disassembled and chromosomes are released, enabling an open mitosis that takes place within the daughter cell (Steinberg et al., 2001; Straube et al., 2005; Theisen et al., 2008). This process is most probably regulated by a conserved signalling pathway containing small GTPase Ras3 and dual-function germinal centre kinase Don3 (Straube et al., 2005; Sandrock et al., 2006).
Nuclear and cytoplasmic microtubule organizing centres (MTOCs, minus-ends of microtubules) nucleate dynamic microtubules that are organized in antiparallel bundles traversing the length of unbudded cells (Straube et al., 2003). During cell division, MTOCs are transported by dynein towards the neck region, where they polarize the microtubule cytoskeleton (Fink & Steinberg, 2006). Growing plus-ends reach into the distal pole of the mother as well as towards the growing pole of the daughter cell (Straube et al., 2003).

One transport function of microtubules is the movement of endosomes containing the t-SNARE-like protein Yup1 (Wedlich-Söldner et al., 2000). These endosomes shuttle bidirectionally along microtubules. Their active transport is mediated by the concerted action of dynein and the plus-end-directed molecular motor Kin3, a Kinesin-3 type motor related to UNC104 and KIF1A from higher eukaryotes (Wedlich-Söldner et al., 2002). Strains carrying a temperature-sensitive mutation in yup1 are altered in cell shape, indicating that yup1-positive endosomes are important for morphology (Wedlich-Söldner et al., 2000). However, inhibition of microtubule function does not cause such morphological alterations (Fuchs et al., 2005), indicating that endosomes might exhibit a microtubule-independent function in regulating morphology during cell growth. Two lines of evidence suggest that endosomal transport is necessary for septation. Firstly, endosomes accumulate at the site of septum formation (Wedlich-Söldner et al., 2000; Schink & Bölker, 2009) and secondly kin3A strains are defective in cytokinesis due to aberrant septum formation (Wedlich-Söldner et al., 2002).

To complete the cell division, mother and daughter cells need to form two septa consecutively (Fig. 1e–g). Between these septa, a vacuolar fragmentation zone is formed, defining the location of the breakdown of the connecting cell wall and the physical separation (O’Donnell & McLaughlin, 1984; Banuett & Herskowitz, 2002; Weinzierl et al., 2002).

Mutants in this process were identified by their aberrant donut-shaped colonies on plates, hence their name don1 and don3 (Weinzierl et al., 2002; Feldbrügge et al., 2006). In a liquid medium, don mutants exhibit a distinct cell separation defect. They form tree-like cell clusters due to their failure to form a secondary septum (Weinzierl et al., 2002). Don1 is a guanine nucleotide exchange factor (GEF) that specifically activates the small G-protein Cdc42 (Weinzierl et al., 2002; Mahlert et al., 2006; Hlubek et al., 2008). Don3 is a dual-function germinal centre kinase that acts parallel to the Cdc42 signalling pathway (Weinzierl et al., 2002; Sandrock et al., 2006). Chemical genetics revealed that Don3 kinase triggers the dynamic rearrangement of septins from hourglass-shaped collars into ring-like structures, depending on a contractile actomyosin ring (Böhmer et al., 2008, 2009). Thus, a signalling network containing small G-protein Cdc42 and kinase Don3...
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is crucial in determining septin and actin-ring organization during secondary septum formation.

Substantial progress has recently been made by connecting components of this signalling network to microtubule-dependent transport (Schink & Bölk, 2009). The authors demonstrate that Don1 is targeted to Yup1-positive endosomes via the specific interaction of the lipid-binding FYVE domain of Don1 with phosphatidylinositol 3-phosphate, a lipid present on these endosomes. Strains expressing a \textit{don1} variant with mutations in the FYVE domain are defective in secondary septum formation like \textit{kin3\Delta} strains. The data suggest that the exchange factor Don1 is targeted towards sites of secondary septum formation by microtubule-dependent transport of endosomes. Thereby, local activation of its cognate G-protein Cdc42 results in a signalling cascade orchestrating secondary septum formation and separation (Schink & Bölk, 2009). Apart from the recycling of pheromone receptors (Steinberg, 2007b), this is a new function for these endosomes in \textit{U. maydis} and fits the general view of endosomes operating as multipurpose platforms (Gould & Lippincott-Schwartz, 2009).

\section*{Mating and pheromone signalling}

Pheromone signalling determining fungal mating belongs to the best-characterized biological signal transduction phenomena studied in eukaryotes (Elion, 2000; Saito, 2010). The main framework was elucidated in \textit{S. cerevisiae} and, in comparison with various other fungi such as \textit{S. pombe} and \textit{Cryptococcus neoformans}, pheromone signalling proved to be an evolutionarily conserved mechanism (Davey, 1998; Bahn et al., 2007). Key components are G-protein-coupled pheromone receptors that recognize cognate peptide pheromones, resulting in the activation of an evolutionarily conserved MAPK module consisting of three kinases (mitogen-activated protein kinase module; MAPKKK, MAPKK and MAPK). The terminal MAPK phosphorylates, among others, transcription factors that determine pheromone-regulated gene expression. A common feature is that the central MAPK pathways crosstalk with other signalling routes to discern appropriate mating conditions such as the nutritional status (Davey, 1998; Saito, 2010).

In \textit{U. maydis} mating of two haploid cells is a prerequisite for infection (Fig. 2), a process that is regulated by the \textit{a} and \textit{b} mating-type loci. The lipopeptide pheromone of one mating partner is perceived by the cognate receptor of the compatible partner (Bölk, et al., 1992; Spellig et al., 1994; Szabó et al., 2002). This elicits an intracellular signal transduction pathway leading to the activation of a defined MAPK module (Fig. 3a; MAPKKK Kpp4, MAPKK Fuz7 and MAPK Kpp2; summarized in Feldbrügge et al., 2006). Direct phosphorylation of the central HMG box transcription factor Prf1 (high mobility group; Hartmann et al., 1996; Müller et al., 1999; Kaffarnik et al., 2003) triggers the expression of a defined set of genes containing pheromone response elements in their regulatory regions (Urban et al., 1996b; Zarnack et al., 2008). Among these are the \textit{a} mating-type genes, \textit{mta12} and \textit{pra12}, enhancing the pheromone response. This feed-forward regulation is further supported by autoactivation of the \textit{prf1} gene at the transcriptional level (Fig. 3b; Hartmann et al., 1999).

Genome-wide transcriptional profiling revealed that MAPK regulation of Prf1 is necessary for the expression of a defined subset of pheromone-responsive genes. Analysis of strains expressing \textit{prf1} variants with mutated MAPK sites showed that in the majority of cases, pheromone responsiveness of target genes was completely lost while in the remaining cases regulation was alleviated. Among the latter are genes encoding the key transcription factors for filamentous growth \textit{bw/Be} as well as \textit{Rbf1} (Scherer et al., 2006; Heimel et al., 2010b). Thus, target genes respond differentially to MAPK phosphorylation of Prf1 (Zarnack et al., 2008) and MAPK signalling via Prf1 prepares the main transcriptional cascade that regulates filamentous growth and further pathogenic development (Heimel et al., 2010b; Wahl et al., 2010b).

In addition, the pheromone-responsive MAPK module is involved in the regulation of the pheromone-induced cell cycle arrest in the G2 phase, which is a Prf1-independent process (Fig. 3a; Garcia-Muse et al., 2003). This ensures synchronization of the cell cycle of both nuclei after cell fusion. Pheromone stimulation as well as transcriptional activation of a constitutively active MAPKKK triggers reduced expression of several genes encoding cell cycle regulators such as B-type cyclin Clb1 or orthologues of polo kinases (Perez-Martin et al., 2006; Zarnack et al., 2008). It is noteworthy that MAPK signalling also promotes a negative feedback loop by inducing the expression of Rok1, a dual-specificity phosphatase that inactivates MAPK Kpp2 (Fig. 3a; Di Stasio et al., 2009).

Moreover, the MAPK module is responsible for eliciting the formation of conjugation tubes, another Prf1-independent event (Fig. 3a; Müller et al., 2003). During the polar growth of these filaments, the pheromone receptor localizes to their tips. This specific localization as well as receptor turnover by endocytosis might be important for the orientation within the pheromone gradient. Thereby, conjugation tubes of compatible mating partners grow towards each other and fuse at their tips (Snetselaar et al., 1996; Fuchs et al., 2006). After plasmogamy, pheromone signalling is switched off by an active \textit{bW/bE} heterodimer (Laity et al., 1995; Urban et al., 1996b; Heimel et al., 2010a).

Mating on the surface of the plant is crucial for infection. Thus, cell recognition and fusion are influenced by environmental factors such as nutrient availability, hydroxy fatty acids or hydrophobic surfaces (Hartmann et al., 1999;
Mendoza-Mendoza et al., 2009a). Two molecular mechanisms for the underlying signalling integration event have been identified. Firstly, an evolutionarily conserved cAMP signalling pathway that consists of a receptor-coupled heterotrimeric G-protein, adenylate cyclase and protein kinase A (PKA) influences mating (Feldbrügge et al., 2006; Brefort et al., 2009). An important molecular mechanism is the direct phosphorylation of Prf1 by cAMP-activated PKA, a regulatory event that is apparently a prerequisite for pheromone response (Fig. 3a; Kaffarnik et al., 2003). PKA-mediated activation of Prf1 is also sufficient to increase the expression of mating-type genes, indicating that upregulation of the intracellular cAMP level favours mating (Kaffarnik et al., 2003; Zarnack et al., 2008). Crosstalk of the PKA and MAPK signalling is also important at later stages of the pathogenic development (Feldbrügge et al., 2006; Brefort et al., 2009).

Secondly, prf1 expression at the transcriptional level is tightly controlled by various regulatory elements in the promoter region (Fig. 3b). For example, the upstream activating sequence (UAS) element integrates different carbon sources (Hartmann et al., 1999) and the unusual MAPK Crk1 signals via this element (Garrido et al., 2004). In addition, the HMG box transcription factor Rop1 as well as the CCAAT-box-binding protein Hap2 are crucial for the activation of prf1 expression via binding sites in the prf1 promoter (Brefort et al., 2005; Mendoza-Mendoza et al., 2009b). Thus, at least four different transcriptional activators (Prf1, yet unknown UAS-binding protein X, Rop1, Hap2) that integrate potentially different signals converge at the prf1 promoter to accurately control the expression of this key transcription factor (Fig. 3b). This is an excellent example of how a central signalling node can integrate various external and internal cues to determine optimal mating conditions.

Filamentous growth and transcriptional networking

Homeodomain transcription factors constitute crucial players in developmental programmes not only in fungi, but also in plants and animals (Pearson et al., 2005; Hay & Tsiantis, 2010). They often function as master regulators eliciting transcriptional cascades that include additional transcription factors. In U. maydis, the homeodomain transcription factor bW/bE is necessary and sufficient to regulate filamentous growth. This Prf1-activated key regulator connects sexual and pathogenic development. Right after its discovery at the b mating-type locus, it was proposed that, like homeodomain transcription factors from higher eukaryotes, it triggers a defined transcriptional programme essential for development (Kronstad & Leong, 1990; Schulz et al., 1990; Gillissen et al., 1992). However, it turned out to be more difficult than expected to identify the responsible target genes. Only very recently a major breakthrough was achieved with the advent of genome-wide transcriptional profiling (Kahmann & Kämper, 2004; Heimel et al., 2010b; Wahl et al., 2010b).

Initially, it was discovered that the activity of the transcription factor was regulated by heterodimerization of subunits derived from different mating partners (Kämper et al., 1995). This information was used to demonstrate that an active bW/bE heterodimer is sufficient to elicit pathogenic development. Haploid strains were generated that express active versions under the control of their own promoters. These strains are called solopathogenic because they infect plants independent of mating (Bölker et al., 1995).

For the identification of functionally important components of filamentous growth, various comparative methods were applied investigating haploid cells and b-induced filaments. Several genes were identified that exhibited significantly increased expression during filamentous growth, but unfortunately, these were all dispensable for infection (Schauwecker et al., 1995; Wösten et al., 1996; Romeis et al., 2000).

A significant improvement was the generation of haploid strains expressing active bW2/bE1 variants under the control of carbon- or nitrogen-source-regulated promoters. For the first time, this allowed a defined time-resolved monitoring of the underlying regulatory cascade (Brachmann et al., 2001). Using RNA fingerprinting, 10 additional targets could be identified (Brachmann et al., 2001). For example, the involvement of MAPK Kpp6 in the early steps of infection such as plant penetration could be demonstrated (Brachmann et al., 2003).

The same strains were the foundation for applying high-density microarrays covering around 90% of all predicted genes (Heimel et al., 2010b). Performing extensive time-course experiments with carbon- and nitrogen-source-mediated activation of the bW/bE heterodimer resulted in the detection of 345 bW/bE-responsive genes (206 induced and 139 repressed; Heimel et al., 2010b). Global analysis of the respective gene functions revealed that 20 genes involved in cell wall remodelling, such as chitin synthases, exochitinases, chitin deacetylases as well as glucanases, are induced, indicating active remodelling of the fungal cell wall during filamentous growth (Heimel et al., 2010b). A number of clustered genes encoding potential effector proteins for plant interaction were also activated (Kämper et al., 2006; Heimel et al., 2010b). Among the downregulated genes are several encoding cell cycle regulators such as cyclins Cin1, Clb1 and Clb2. For Cin1, it has been shown that repression leads to G2 cell cycle arrest (Garcia-Muse et al., 2004). Apparently, the bW/bE heterodimer is responsible for maintaining the G2 cell cycle arrest until the filament...
Development of the pathogen U. maydis resumes the cell cycle after plant penetration (Perez-Martin et al., 2006).

One of the most rewarding results of this analysis was the identification of the downstream master regulator Rbf1. This zinc finger transcription factor, which is an immediate early target of the bW/bE heterodimer at the transcriptional level, is responsible for the vast majority of the gene expression programme (Fig. 4a; Heimel et al., 2010b). Accordingly, overexpression of Rbf1 is sufficient to trigger filamentous growth. Because it is difficult to envision that hundreds of different bW/bE heterodimers trigger the same transcriptional programme, the presence of such a master regulator was no surprise. Interestingly, rbf1 is also a direct Prf1 target and its expression is pheromone induced via MAPK signalling (Fig. 4b; Zarnack et al., 2008). Thus, during the initial phase of mating, Rbf1 activity must be inhibited by a currently unknown mechanism.

Rbf1 amplifies the transcriptional cascade by the activation of various transcriptional regulators such as Biz1 and Fox1 (Fig. 4a; Heimel et al., 2010b). Biz1 is a zinc finger-containing factor involved in cell cycle regulation and is important for appressoria formation and plant penetration (Flor-Parra et al., 2006). Fox1 regulates the expression of numerous effector proteins that appear to be involved in the suppression of plant defence (Zahiri et al., 2010).

Using a temperature-sensitive mutant, it was demonstrated that the active bW/bE transcription factor is not only needed to establish the transcriptional cascade of pathogenic development, but is also crucial at later stages. Interfering with the activity of bW/bE in planta results in an unusual activation of pheromone signalling, defects in cell cycle synchronization and reduced expression of effector proteins. This aberrant regulation correlates with impaired infection (Wahl et al., 2010b).

Another important direct target of the bW/bE transcription factor is clp1 (Fig. 4b). It encodes a regulator that is required for clamp formation, a special structure needed for nuclear sorting during mitotic division of the dikaryon in planta (Scherer et al., 2006). Recently, the level of complexity was added to by demonstrating that Clp1 interacts with bW and counteracts bW/bE-dependent functions such as G2 cell cycle arrest and filamentous growth (Fig. 4b). Clp1 also interacts with Rbf1 during the repression of pheromone signalling (Heimel et al., 2010a). Furthermore, Clp1 binds Cib1, a bZIP transcription factor required for pathogenic development, whose activity is not regulated at the transcriptional level, but at the level of alternative splicing (Fig. 5b; Heimel et al., 2010a). Thus, besides the simple linear transcription cascade Prf1 > bW/bE > Rbf1 (Fig. 4a), there is an underlying complicated network of transcription factors and protein interactions that mediate intensive feedback and feedforward regulation to orchestrate pathogenic development (Fig. 4b).

Filamentous growth and cytoskeletal functions

In general, fungal hyphae exhibit a characteristic growth mode. At their apical pole, a growth cone is responsible for the rapid expansion of membrane and cell wall. In subapical regions, filaments are partitioned by septa forming molecular barriers (Harris, 2006; Steinberg, 2007a; Fischer et al., 2008; Zarnack & Feldbrügge, 2010). Polar growth is mediated by extensive membrane dynamics, i.e. local exo- and endocytosis that is supported by vesicle trafficking. This might be promoted by distinct membrane microdomains at the apical pole that are rich in specific lipids such as sphingolipids and ergosterol (Harris et al., 2005; Steinberg, 2007b). Two juxtaposed macromolecular units are involved in growth cone function: the Spitzenkörper and the polarisome. The latter is a multiprotein complex consisting of landmark proteins, signalling molecules such as small GTPases and forms that nucleate actin cables for short-distance transport (Harris et al., 2005; Sudbery & Court, 2007). The Spitzenkörper is thought to function as a supply centre for exocytotic vesicles (Gierz & Bartnicki-Garcia, 2001; Bartnicki-Garcia, 2002), for example, specialized vesicles, so-called chitosomes, transport and export chitin synthases as well as building blocks for cell wall synthesis (Bartnicki-Garcia, 2006; Riquelme et al., 2007). Active transport along the actin and microtubule cytoskeleton is mediated by molecular motors for short- and long-distance delivery of vesicles, respectively. This process is streamlined for efficiency and any disturbances lead to reduced growth rates or a complete blockage (Harris, 2006; Steinberg, 2007a).

In U. maydis, a number of recent observations confirm that dikaryotic filaments grow like typical hyphae (Steinberg, 2007a). For example, the landmark protein Spa2 localizes to the tip (Carbo & Perez-Martin, 2008) and sphingolipid biosynthesis is important for polar growth. These lipids are most likely part of membrane microdomains at the hyphal tip (Canovas & Perez-Martin, 2009) and could function to enrich signalling components with membrane anchors such as the small G protein Rac1 (Mahler et al., 2006). Septins might serve as diffusion barriers organizing these membrane subdomains. Strains carrying corresponding loss-of-function mutations are affected in establishing the axis of polarity, whereas septins are dispensable during the maintenance of filamentous growth (Alvarez-Tabares & Perez-Martin, 2010).

Analysis of eight different chitin synthases revealed that Chs7 was particularly important for polar growth and localizes to the hyphal tip like its related family members Chs5 and Mcs1 (Weber et al., 2006). The chitin synthase Mcs1 contains a unique domain with a similarity to myosin motors (Weber et al., 2006). This domain is not required for motility, but apparently for different functions (Treitschke.
Polar growth (Weber et al., 2007a). The actin-dependent motor Myo5 is potentially involved in polar growth. Accordingly, the motor accumulates at plus-ends of actin cables at the poles of filaments, and loss of Myo5 results in severe defects during polar growth (Weber et al., 2003; Schuchardt et al., 2005).

Long-distance transport of molecular cargo is mediated by the microtubule cytoskeleton (Steinberg, 2007c). Although microtubules are dispensable for filamentous growth, they are needed to determine the axis of polarity. In their absence, growth is retarded and defects in the insertion of basal retraction septa occur (Fuchs et al., 2005; Steinberg, 2007a). One of the main functions of microtubules during filamentous growth appears to be the bidirectional transport of Yup1-positive vesicles that also colocalize with the endosomal marker Rab5, a small GTPase (Fuchs et al., 2006). Although the knowledge about the function of endosomal shuttling is currently scarce, significant progress has been made in analysing and modelling the mechanism of transport by molecular motors (Steinberg, 2007c; Ashwin et al., 2010; Schuster et al., 2011a). Study of plus-end-binding proteins revealed that microtubules are highly polarized, with plus-ends localizing to both poles (Lenz et al., 2006). Similar to haploid cells, Kin3 and split Dyn1/2 mediate anterograde and retrograde transport of endosomes, respectively (Lenz et al., 2006; Schuster et al., 2011b). Dynein as well as its regulators dynactin and Lis1 accumulate at the plus-ends of microtubules, where they might form a reservoir for retrograde transport (Lenz et al., 2006; Schuster et al., 2011a). Apparently, dynein can be loaded onto endosomes either at plus-ends of microtubules or during its retrograde movement (Schuster et al., 2011a,b). Conventional kinesin Kin1 (Lehmler et al., 1997) is responsible for recycling dynein to the microtubule plus-ends for additional rounds of minus-end-directed transport (Becht et al., 2006; Lenz et al., 2006). Notably, kin1 was initially named kin2 in a study describing kin1 and kin2 encoding a Kinesin-7 and Kinesin-1 type motor, respectively (Lehmler et al., 1997). In order to apply a standardized kinesin nomenclature, kin1 and kin2 were subsequently renamed kin7a and kin1, respectively (Lawrence et al., 2004; Schuchardt et al., 2005).

As mentioned above, knowledge of the biological function of endosomal transport during polar growth is still limited. It has been speculated that these vesicles constitute early endosomes that might be important for endocytotic membrane recycling or act during long-distance signalling to the nucleus (Fuchs et al., 2006; Steinberg, 2007a). However, additional work is required to substantiate these assumptions.

**Filamentous growth and post-transcriptional regulation**

Post-transcriptional regulation is essential in determining the quantitative and spatiotemporal expression of proteins. Key factors are RNA-binding proteins that function, for example, during the splicing, localization, stability and translation of mRNAs (Dreyfuss et al., 2002). These processes can also be coupled. Localization and translation of mRNAs result, for example, in the defined subcellular localization of the encoded protein, a cellular mechanism...
that is conserved from bacteria to humans (St Johnston, 2005; Du et al., 2007; Nevo-Dinur et al., 2011). mRNA localization is mainly achieved by active transport along the actin or the microtubule cytoskeleton. In S. cerevisiae, ASH1 mRNA is actively transported from the mother cell towards the distal pole of the daughter cell. Local translation at the pole ensures that the encoded transcription factor specifically localizes to the nucleus of the daughter cell in order to ascertain a distinct transcriptional programme (Jansen, 2001; Zarnack & Feldbrügge, 2007). ASH1 mRNA transport is mediated by two RNA-binding proteins, She2p and She3p, that cooperatively bind RNA elements, so-called zipcodes, in the target mRNA. This is essential for actin-dependent transport mediated by myosin (Bohler et al., 2000; Müller et al., 2011). Recently, it was reported that She3p-mediated ASH1 mRNA transport is also operational in filamentous Candida albicans, most likely to regulate hyphal morphology and invasive growth (Elson et al., 2009).

Microtubule-dependent mRNA transport is mainly studied in higher eukaryotes. Important processes regulated at this level are oocyte and embryo development in Drosophila melanogaster as well as neuronal processes in mammals (St Johnston, 2005; Dahm et al., 2007). Although the RNA-binding proteins and the transported target mRNAs are evolutionarily not conserved, the general concept holds true for all investigated transport processes to date: specific RNA-binding proteins recognize zipcodes forming large mRNPs (mRNA-containing ribonucleoprotein complexes) that are transported along microtubules by kinesin(s) and dynein (Holt & Bullock, 2009).

The importance of such control at the post-transcriptional level is an emerging theme for U. maydis. This additional layer of regulation functions in concert with the extensive regulation at the level of transcription described above (Feldbrügge et al., 2008; Vollmeister & Feldbrügge, 2010). For example, although Clp1 is a direct and immediate early target gene of bW/bE, the protein accumulates only at later stages during plant penetration, suggesting translational control (Scherer et al., 2006). Moreover, precise expression of the Clp1 interaction partner Cib1 appears to be regulated by alternative splicing (Heimel et al., 2010a).

Investigating various RNA-binding proteins in U. maydis revealed that two of them, Khd4 and Rrm4, are important for filamentous growth (Becht et al., 2005). The loss of Khd4 causes pleiotropic effects such as altered cell and filament morphology as well as defects in pheromone response and plant infection (Becht et al., 2005). The protein contains at least five potential RNA-binding domains of the KH type (hnRNP K homology domain, KH1-5). It constitutes the founding member of a multi-KH domain family of fungal proteins with representatives in other pathogens such as C. neoformans and C. albicans. Khd4 recognizes the sequence AUACCC via the two central tandem-KH domains (KH3 and KH4; Vollmeister et al., 2009). Interestingly, this motif is enriched in the 3′ untranslated region of mRNAs and a number of transcripts, which exhibit altered expression in khd4Δ strains, contain this binding site. Because most mRNAs show an increased abundance in deletion strains, Khd4 might promote mRNA instability (Vollmeister et al., 2009; Vollmeister & Feldbrügge, 2010). Direct target mRNAs, whose expression is regulated by Khd4 and whose function can be related to the mutant phenotypes, are yet to be identified. To this end, we are currently conducting in vivo UV crosslinking experiments (König et al., 2010).

The deletion of rrm4 causes specific defects during filamentous growth, namely bipolar growth and failure to insert retraction septa (Fig. 5a–c; Becht et al., 2005, 2006). Interestingly, this mutant phenotype is reminiscent of growth defects of strains affected in microtubule functions (Lehmler et al., 1997; Steinberg et al., 1998; Fuchs et al., 2005; Schuchardt et al., 2005; Schuster et al., 2011a). Rrm4 contains three N-terminal RNA recognition motifs and a C-terminal MLLE domain that mediate RNA binding (Bacht et al., 2006; König et al., 2007) and protein/protein interactions, respectively (Kozlov et al., 2010). Subcellular localization revealed that Rrm4 shuttles in mRNPs along microtubules (Fig. 5d–e; Supporting Information, Movies S1 and S2). RNA binding as well as the formation of motile mRNPs are essential for protein function (Bacht et al., 2006; König et al., 2007). The deletion of kin1 results in the accumulation of Rrm4 at the poles. This is consistent with the hypothesis that split dynein mediates the retrograde transport of mRNPs, because conventional kinesin mediates anterograde transport of split dynein to the plus-ends of microtubules (Bacht et al., 2006; S. Baumann, T. Pohlmann & M. Feldbrügge, unpublished data). In vivo UV crosslinking revealed that Rrm4 binds a distinct set of mRNAs that contain a potential CA-rich binding motif (König et al., 2009). These mRNAs encode proteins of cytotopically related groups such as proteins involved in translation, mitochondrial proteins or polarity factors. Important examples are ubi1 and rho3 encoding a natural fusion of ubiquitin with ribosomal protein Rpl40 and a small GTPase, respectively (König et al., 2009). RNA live imaging revealed that these mRNAs are molecular cargos of the Rrm4 transport unit. Remarkably, the CA-rich 3′ UTR of ubi1 functions as a cis-active region in increasing the amount and processivity of trafficking (König et al., 2009). This is a characteristic feature of so-called mRNA zipcodes. Rho3 accumulates at retraction septa, suggesting a regulatory role during this process. These data led to the current model that Rrm4 functions in long-distance transport of mRNAs, a process that is conserved throughout evolution (Fig. 5f–g; Zarnack & Feldbrügge, 2007, 2010). The local translation of Rrm4 target mRNAs appears to be important for the subcellular...
localization of encoded proteins (Fig. 5f–g). Intriguingly, we could recently show that Rrm4-containing mRNPs are cotransported with Yup1-positive endosomes, suggesting yet another function of microtubule-dependent transport of endosomes (discussed above; S. Baumann, T. Pohlmann & M. Feldbrügge, unpublished data).

**Filamentous growth and mitochondrial inheritance**

Sexual reproduction in eukaryotes goes along with the combination of nuclear and organelle genomes, such as mitochondrial genomes, in the zygote. This allows recombination events to accelerate evolution. However, a common process in eukaryotic sex is uniparental mitochondrial inheritance, a process that results in the asexual inheritance of the organelle genome. A possible explanation is the avoidance of evolutionary conflicts caused by a heteroplasmic situation. For example, mitochondria with increased replication rates, but decreased functional performance might dominate the population, resulting in disadvantages for the zygote (Partridge & Hurst, 1998; Xu, 2005). Possible mechanisms for uniparental mitochondrial inheritance include unequal size and mitochondrial numbers of gametes (Xu, 2005). An extreme example is human oocytes carrying about 10 000 more mitochondrial genomes than sperm cells, providing for a confident head start of maternal mitochondria in the population. Other mechanisms operating post-fusion of the gametes include the interplay with nuclear-encoded genes (Basse, 2010). For example, the mating-type specific homeodomain genes control uniparental inheritance in *C. neoformans*, suggesting that respective target genes encode proteins that determine uniparental inheritance (Xu, 2005; Yan et al., 2007).

In *U. maydis*, the inheritance of mitochondria is determined at late stages of filamentous growth when the fungus penetrates the plant, and mitochondria are passed on to the next cell. Importantly, uniparental mitochondrial inheritance exists in *U. maydis* and the a2 mitotype is predominantly inherited (Fedler et al., 2009). How is this achieved at the molecular level? Initially, it was observed that the a2 mating-type locus, but not its a1 counterpart contains two genes, lga2 and rga2 (left and right genes of the a2 locus), which are only found in *U. maydis* and close relatives such as the head smut fungus *Sporisorium reilianum* (Urban et al., 1996a; Schirawski et al., 2005b). Because Lga2 contains a mitochondrial import signal, it was proposed that it might be involved in uniparental mitochondrial inheritance (Urban et al., 1996a). Work accomplished in the last few years has supported this hypothesis and has shed some light on the underlying mechanism (Basse, 2010).

During mating conjugation tubes fuse, plasmogamy occurs and cellular contents including the mitochondria are mixed (Fig. 2). How is it possible to separate the two mitochondrial populations to ensure uniparental inheritance? The key player is Lga2, a protein that is attached to the outside of the mitochondria (Bortfeld et al., 2004; Mahlert et al., 2009). This protein serves two main functions. Firstly, it inhibits the fusion of the mitochondria (Fig. 6) to prevent the mobilization of homing introns and secondly, Lga2 triggers mitochondrial fragmentation and mtDNA degradation (Bortfeld et al., 2004; Mahlert et al., 2009). Rga2 also localizes to the mitochondria even though a clear targeting signal is missing (Bortfeld et al., 2004). It appears to counteract Lga2 by protecting the mitochondria of the a2 mitotype (Fedler et al., 2009; Basse, 2010).

Based on the expression of these genes, the following model can be proposed (Basse, 2010). Like in all genes of the a locus, lga2 and rga2 expression is activated upon pheromone signalling (Urban et al., 1996b). Thereby, a2 mitotype mitochondria are preloaded with Lga2 and Rga2 before cell fusion. In contrast to *mfa1/2* and *pra1/2*, which are repressed by an active bW/bE heterodimer, lga2 is one of the few directly activated targets of the bW/bE regulator, and

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**Fig. 5.** Microtubule-dependent shuttling of Rrm4-containing mRNPs is important for filamentous growth. (a) Filament of a monokaryotic strain expressing active bW2/bE1 variants under the control of a nitrogen-source-regulated promoter. Filaments are grown for 6 h under inducing conditions. Asterisk marks the predicted position of the next retraction septum (size bar = 10 µm). (b) *rrm4Δ* cells show no aberrant growth phenotype. (c) *rrm4Δ* filament exhibits defects in the formation of a single axis of polarity. The initial cell forms two growth cones and fails to insert a retraction septum (Becht et al., 2006). (d) Filament expressing Rrm4 fused to Gfp. The fusion protein accumulates in defined cytoplasmic particles (arrowheads in the inverted image detecting Gfp fluorescence) that shuttle bidirectionally along microtubules (kymograph in the lower part). In the kymograph, time is plotted vs. distance. Thus, motion of Rrm4 particles is visible as defined tracks (note the reversal of shuttling at the poles). Picture and kymograph correspond to Movie S1. (e) Filament expressing Rrm4 fused to Gfp was treated with the microtubule inhibitor benomyl (Fuchs et al., 2005). Presented as described in (d). Picture and kymograph correspond to Movie S2. (f) Model depicting microtubule-dependent transport of mRNAs. Rrm4-containing particles (dark red circles) transport mRNAs (red wavy lines) carrying the poly(A)-binding protein (blue ovals) bidirectionally along microtubules (black lines). The transport of rho3 mRNA might promote the accumulation of Rho3 at the retraction septum. (g) Deletion of *RRM1* to *RRM3* in *rrm4* causes a loss-of-function phenotype. Filaments grow mostly bipolar and target mRNAs are no longer transported along microtubules. The mutant *rrm4* is still part of the shuttling units, indicating that *rrm4* is an integral component of the transport machinery and does not just hitchhike like the poly(A)-binding protein ([f], [g] are reprinted from Zarnack & Feldbrügge (2010) with permission from the American Society of Microbiology).
rga2 expression is maintained constant (Romeis et al., 2000; Brachmann et al., 2001). Consistently, early during plasmogamy, Lga2 prevents the fusion of mitochondria (Fig. 6) and over time Lga2 eliminates those mitochondria that are not sufficiently protected by Rga2, namely mitochondria of the a1 mitotype (Basse, 2010).

Possible downstream effectors for the two functions of Lga2 are dynamin-related GTPase Dnm1 and the mitochondrial protein Mrb1. Dnm1 is crucial in preventing mitochondrial fusion in an Lga2-dependent manner (Mahlert et al., 2009). Mrb1, a regulator of the p32 family, interacts with Rga2 and appears to counteract the function of Lga2 (Bortfeld et al., 2004; Basse, 2010). This is based on the following observation. While a2mrb1Δ strains fail to infect plants, virulence can be restored by the deletion of lga2. Importantly, dikaryons of a1a2mrb1Δ strains are impaired in virulence because they are arrested at the early phase of infection when uniparental inheritance should occur (Bortfeld et al., 2004). However, its precise mode of action is currently unclear.

This is now the first glimpse of this process, but as so often, the story is more complex. For example, the dominance of a2 mitotypes is not 100%, indicating additional mechanisms independent of the Lga2/Rga2 system (Bortfeld et al., 2004; Fedler et al., 2009). Moreover, in S. reilianum, there are three mating types: a1, a2 and a3. Lga2 and rga2 are present on a2, but successful mating and potential uniparental mitochondrial inheritance is possible between a1 and a3 (Schirawski et al., 2005b).

Plant infection and fungal effector proteins

For successful infection, mating should take place on the plant surface, such as on the leaves, stems or part of the flower. Therefore, it is not surprising that plant-derived signals stimulate the formation of filaments as well as appressoria (Fig. 7). For example, corn lipids trigger filamentous growth in liquid culture (Klose et al., 2004). Moreover, hydrophobicity acts as an inducer of filamentation and appressorium differentiation on artificial surfaces and the latter is further enhanced by the addition of hydroxy fatty acids. Because both signals are present on the plant surface, they are most likely also operational under natural conditions (Fig. 7; Mendoza-Mendoza et al., 2009a).

Two signalling components, the tetraspan membrane protein Sho1 and its interaction partner the single transmembrane mucin Msb2, appear to be involved in sensing the hydrophobic signal. Accordingly, the deletion of the corresponding genes strongly reduces appressoria formation. Sho1 interacts specifically with MAPK Kpp6, suggesting that Sho1/Msb2 operate upstream of a Kpp6-containing MAPK module during early infection (Lanver et al., 2010).

The perception of host signals is not the only crucial event that is a prerequisite for the development of infection structures on the plant surface. Additional processes involve actin-mediated functions (Berndt et al., 2010) as well as components of the N- and O-glycosylation pathways (Schirawski et al., 2005a; Fernandez-Alvarez et al., 2010). Unfortunately, insights into the underlying mechanisms that influence early infection are currently missing.

Once biotrophic fungi enter the plant, they depend on living material for growth and nutrition, so that many biotrophs form specialized feeding structures termedhaustoria (Mendgen & Hahn, 2002). During plant invasion of U. maydis fungal hyphae invaginate the plasma membrane of infected host cells, leading to the formation of a biotrophic interface without extensive feeding structures. Instead, hyphae orient their growth towards vascular bundles, where they might feed on transported sugars (Doehlemann et al., 2008a; Brefort et al., 2009). In line with this, a novel sucrose transporter, Srt1, was recently described as a major component ensuring fungal nutrition (Fig. 7; Wahl et al., 2010a).

Interestingly, this transporter has a higher substrate affinity than a comparable plant transporter, suggesting a redirection of sugar flow towards the parasite (Wahl et al., 2010a).

The invasion and proliferation of pathogens within a plant are often accompanied by host recognition, usually triggering defence. Thus, for survival, a successful biotroph

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**Fig. 6.** Separate parental populations of mitochondria in dikaryotic filaments. Tip of dikaryon resulting from the mating of strain FB1 (a1b1) with GFS (a2b13). These strains express Gfp and Rfp targeted to the mitochondria, respectively (mtGfp and mtRfp). Epifluorescence images show green and red fluorescence as well as a merged picture at the bottom, indicating that mitochondria of the two parental cells do not fuse during filamentous growth [figure reprinted from Fedler et al. (2009) with permission from the Genetics Society of America].
should avoid or suppress plant immune responses. Indeed, transcriptional profiling of infected maize indicated that *U. maydis* is recognized by the plant within the first 24h after infection as, for example, pathogenesis-related genes are activated. At later stages, however, plant defence appears to be specifically shut down (Fig. 7; Doehlemann et al., 2008a). A possible mechanism would be the interference with plant signalling. Conceivably, jasmonic acid signalling, which appears to be induced in infected tissue, antagonizes salicylic acid signalling, a key component of the plant defence against biotrophs (Doehlemann et al., 2008a).

In accordance with the current views, the fungal pathogen uses secreted effectors to trigger alterations of plant responses during biotrophic growth. Effectors are delivered to the biotrophic interface that constitutes a hotspot for plant–pathogen communication. One important finding that went along with the completion of the genome sequence was the discovery of gene clusters coding for novel secreted proteins that are crucial for infection (Fig. 7; Kämper et al., 2006). In five of 12 cases, the deletion of distinct gene clusters led to disease-associated phenotypes, indicating that these clusters contain virulence factors (Kämper et al., 2006). A common feature is that clustered effector genes exhibit strong transcriptional activation during biotrophic growth (Kämper et al., 2006), and many of these genes with virulence-associated phenotypes are regulated in a bW/bE-dependent manner (Wahl et al., 2010b). Apart from clustered effector genes, there are also single genes and gene families scattered throughout the genome that encode potential effectors (Kämper et al., 2006; Doehlemann et al., 2009; Khrunyk et al., 2010). Currently, the best-studied example is Pep1, an effector that is essential for biotrophic development. pep1 deletion mutants arrest during penetration without causing disease symptoms (Doehlemann et al., 2009). They fail to induce jasmonic acid signalling and do not suppress the plant defence mechanism during the early stage of infection. This is accompanied by an accumulation of toxic substances and papillae formation at the penetration sites (Doehlemann et al., 2009).

Remarkably, it appears that specific effector subsets are important during different stages of pathogenic development, as the block of corresponding mutants differed with respect to the infection stage (Kämper et al., 2006). This finding was recently expanded by providing evidence for organ-specific effector functions. Because plant tissues and organs differ strongly in their physiology, the fungus might adapt to each environment using a special, spatiotemporal-regulated assembly of effectors (Skibbe et al., 2010). Recently, the genome of *S. reilianum*, a related corn pathogen, was sequenced and annotated (http://mips.helmholtz-muenchen.de/genre/proj/sporisorium; Schirawski et al., 2010). Genome mining revealed that many of the genes for novel secreted proteins found in *U. maydis* are also present. This is likely due to the fact that they parasitize on the same host. However, most
shared effectors are highly divergent, which may be attributed to the differing infection strategies of *U. maydis* and *S. reilianum* (Schirawski et al., 2010). In essence, during development in planta *U. maydis* relies on numerous, thoroughly regulated effectors to induce the host to foster its development.

Conclusions

Our tour following the fungal development of *U. maydis* revealed quite a number of remarkable recent findings. Highlights are for example (1) the endosomal transport of signalling components during cytokinesis (Schink & Bölker, 2009); (2) the discovery of Rbf1 and the underlying extensive regulation at the transcriptional and post-transcriptional level (Heimel et al., 2010a,b); (3) uniparental mitochondrial inheritance (Fedler et al., 2009); (4) microtubule-dependent mRNA transport (König et al., 2009); and (5) fungal effectors during infection (Kämper et al., 2006; Brefort et al., 2009).

In the future, resolving the underlying molecular mechanisms will be essential in answering the following key questions: how do endosomes function during membrane recycling, signalling and microtubule-dependent transport? How is the expression of transcription factors regulated at the post-transcriptional level? What are the precise molecular functions of effectors?

The results obtained will further advance *U. maydis* as a simple eukaryotic model for various aspects of basic cellular functions. Within the last few years, a few scientists have spearheaded distinct research areas covering several aspects of cell biology and plant pathogenicity. Now appears to be the right time for expansion.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Movie S1.** Rrm4-Gfp fusion protein moves bidirectionally along cytoskeletal tracks in filaments of *Ustilago maydis* strain AB33 (150-ms exposure time, 70 frames, five frames/second display rate; quick time format, 9565 kb).

**Movie S2.** Disturbed movement of Rrm4-Gfp fusion protein after treatment with microtubule inhibitor benomyl in filaments of *Ustilago maydis* strain AB33 (150-ms exposure time, 70 frames, five frames/second display rate; quick time format, 102 kb).

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