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Title	The importance of subclasses of chitin synthase enzymes with myosin-like domains for the fitness of fungi
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Abstract	Chitin represents one of the most important components of the fungal cell wall. The multiplicity of chitin synthase (Chs) enzymes found in filamentous fungi underlines the importance of chitin in these organisms. Among this group of fungal enzymes, two classes, V and VII, are armed with myosin motors, constituting the MMD-Chs þÿ (M y o s i n M o t o r D o m a i n C h i that are found in filamentous fungi and are absent in most yeast species. These enzymes play a critical role in promoting the synthesis of chitin at the hypha tip, thus influencing fungal growth and the architecture of fungal infection structures. Other processes in which these enzymes are important are in osmo- and H2O2-tolerance, the ability to grow at 37º C and in conidiogenesis. This review is focussed on the classification, structure and function of these enzymes describing the fundamental role of these enzymes in the ability of filamentous fungi to infect plants and their possible involvement in infections of animals. Moreover, data obtained with deletant mutants of this family of proteins indicates that they have potential

	as targets for novel antifungals.
Keywords	Chitin synthase; Class V; Class VII; myosin-like domain; filamentous fungi
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Dear Editor, Professor Nick Read

On behalf of all the authors I'm submitting to your consideration an original review entitled "**The importance of subclasses of chitin synthase enzymes with myosin-like domains for the fitness of fungi** ".

This reviews spans the literature of saprophytes and pathogens of plants and animals and as such is of interest to the broad readership of Fungal Biology Reviews. We look forward to your comments and those of the reviewers.

Yours sincerely,

Teresa Gonçalves

Dear Editor, Professor Geoffrey Robson

As requested by the reviewers we proceed with the revision of the manuscript entitled "**The importance of subclasses of chitin synthase enzymes with myosin-like domains for the fitness of fungi** ", that we now submit for your consideration. Since the comments were marked in the text of the manuscript we also added our responses point-by-point in the manuscript.

Please let me know if the reviewers ask for a separate document with the responses to their comments and suggestions.

We look forward to your comments and those of the reviewers.

Yours sincerely,

Teresa Gonçalves

Highlights

- Fungal chitin synthases with myosin-like domain (Chs-MMD) are virulence determinants
- These enzymes play a critical role in the synthesis of chitin at the hypha tip.
- Rather than intrahyphal transport, MMD may retain the enzyme in the apical region.
- Chs-MMD play a role in the ability to grow at 37º C, in osmo- and H_2O_2-tolerance.
- Lack of Chs-MMD changes host immune responses.

The importance of subclasses of chitin synthase enzymes with myosin-like domains for the fitness of fungi

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Short Title: Fungal class V and VII Chs (MMD-CHs)

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Abbreviations

Chs	Chitin synthase
MMD-Chs	Myosin Motor Domain – Chitin synthases

Abstract

Chitin represents one of the most important components of the fungal cell wall. The multiplicity of chitin synthase (Chs) enzymes found in filamentous fungi underlines the

importance of chitin in these organisms. Among this group of fungal enzymes, two classes, V and VII, are armed with myosin motors, constituting the MMD-Chs (Myosin Motor Domain – Chitin synthases) that are found in filamentous fungi and are absent in most yeast species. These enzymes play a critical role in promoting the synthesis of chitin at the hyphal tip, thus influencing fungal growth and the architecture of fungal infection structures. Other processes in which these enzymes are important are in osmo- and H_2O_2-tolerance, the ability to grow at 37º C and in conidiogenesis. This review is focussed on the classification, structure and function of these enzymes describing the fundamental role of these enzymes in the ability of filamentous fungi to infect plants and their possible involvement in infections of animals. Moreover, data obtained with deletant mutants of this family of proteins indicates that they have potential as targets for novel antifungals.

Keywords:

Chitin synthase; Class V; Class VII; myosin-like domain; filamentous fungi

1. Introduction

Chitin is a linear β-1,4-linked homopolymer of *N*-acetylglucosamine and is a common component of the walls and exoskeletons of fungi and invertebrates. This component represents only 1 to 2 % of the cell wall dry weight of cell walls of yeast like *Saccharomyces* spp. and is present primarily at the mother-daughter septal junctions (Cid *et al.*, 1995). Chitin constitutes a much bigger fraction of the cell wall (10 to 20% of the cell wall dry weight) of the filamentous fungi mycelia (Bartnicki-Garcia, 1968). In these organisms, chitin is distributed throughout the lateral cell wall with higher deposition at the hyphal tips and at septa (Munro and Gow, 2001; Klis, 1994). In most

species of fungi chitin is critically important for cell wall rigidity (Ruiz-Herrera *et al.*, 2002) and mutants with very low chitin content or lacking specific chitin synthases can be non-viable (Bulawa, 1993; Munro and Gow, 2001).

Along with cellulose, chitin is one of very few linear molecules in nature. Chitin forms extremely strong fibrous microfibrils that are “stronger, weight-for-weight, than bone or steel” (Lenardon, 2010). Such rigidity is achieved because during synthesis, the nascent chitin chain folds back on itself to form anti-parallel chains, which form intra-chain hydrogen bonds that make the carbohydrate stiffer. Chitin is also covalently attached to the β-glucan component of the cell wall, establishing a highly interlinked cell wall structure that is the common basis of the cell wall skeleton of most fungal species. Chitin is essential for polarized cell wall synthesis, maintenance of cell wall integrity (Horiuchi *et al.*, 1999; Kong *et al*., 2012) and for the virulence properties of many pathogenic fungi (Liu *et al.*, 2004; Madrid *et al.*, 2003; Weber *et al.*, 2006; Kong *et al.*, 2012; Lee *et al.*, 2012).

The chitin polymer is synthesized by a set of integral membrane proteins termed chitin synthases (Chs) that obtain their substrate, UDP-*N*-acetylglucosamine, at the cytoplasmic side of the plasma membrane and synthesize linear chains of β(1,4)-GlcNAc, which are transported through the membrane to the external side, where they fold and become cross-linked to other cell wall components (Abramczyk and Szaniszlo, 2009; Choquer *et al.*, 2004; Riquelme, 2013).

Chs are ancient enzymes whose catalytic activity has been conserved during evolution (Ruiz-Herrera *et al.*, 2002). All Chs are transmembrane proteins with the catalytic domain located on the cytoplasmic face. They are grouped into seven classes according to protein sequence similarities (Choquer *et al.*, 2004). These classes are divided into groups on the basis of their amino acid sequence. Classes I, II and III belong to Division

, while Division 2 contains the classes IV, V and VII. The class VI Chs enzymes are unique members of the Division 3 (Choquer *et al.*, 2004; Latgé, 2007; Martín-Udíroz *et al.*, 2004; Sánchez-León *et al.*, 2011) (Fig. 1).

Chs enzymes that fall in Division 1 share a common protein organization composed by the catalytic domain surrounded by a hydrophilic N-terminus region and a hydrophobic C-terminus region. The catalytic site is bordered, on either side, by transmembrane regions, (Bowen *et al.*, 1992; Ruiz-Herrera *et al.*, 2002; Choquer *et al.*, 2004). Whilst, the Chs members of the Division 2 display a similar catalytic domain preceded by a cytochrome b_5-like domain and the central protein core is bound to the membrane through multiple helices at the C-terminus. In this review we focus on class V and VII enzymes that hold a DEK C-terminal domain and an N-terminal myosin motor-like domain (MMD) (Din *et al.*, 1996; Zujiwara *et al.*, 1997; Choquer *et al.*, 2004). Several sub-divisions of classes V and VII Chs have been proposed, based on sequence. We will follow the classification suggested by Roncero and co-workers (2002) in which a Chs that possess a MMD (MMD-Chs) is classified within class V or VII (Niño–Vega *et al.*, 2004; Roncero, 2002) according to their structural characteristics. The MMDs of class V Chs (around 800 amino acids) are longer than those of the class VII Chs (around 600 amino acids). Moreover, the class VII MMD does not have the consensus motifs of myosins, such as the P-loop, Switch I and Switch II (Takeshita *et al.*, 2006; Cheney and Mooseker, 1992) (Fig. 2).

The Chs from Division 3 hold conserved catalytic sequences but do not display any of the characteristics of the protein family encountered in the other Chs (Latgé, 2007). Most of the filamentous fungi hold 10 or more Chs isoenzymes spread among all classes of two divisions. In contrast, yeasts like *Saccharomyces cerevisiae* and *Candida*

albicans contain a reduced number of Chs isoenzymes that fall within classes I, II and IV (Fig. 1) (Lenardon *et al.*, 2010).

The MMD-Chs hybrids are not exclusive to fungi. They are also found in molusks (Weiss *et al.*, 2006). Other myosin hybrids have also been reported, such as the kinase/myosin hybrid present in *Drosophila* (Mooseker and Foth, 2008). Filamentous fungi and some dimorphic fungi tend to carry only one Chs from each of the classes V and VII. Some exceptions are known, such as in the yeast *Yarrowia lipolytica* with 7 *CHS* genes, three of which contain MMD, one class V and two class VII (Sheng *et al.*, 2013). In contrast, *Ustilago maydis* and *Cryptococcus neoformans* carry two class V Chs, one MMD-Chs and one other depleted of the MMD (Garcerá-Teruel *et al.*, 2004; Banks *et al.*, 2005). Interestingly, *C. neoformans* normally grows as yeast during infection, saprophytic life, or under normal laboratory culture conditions (Zaragoza *et al.*, 2009; Fu *et al.*, 2013). Since MMD-Chs are confined to filamentous fungi, it has been suggested that they are of importance for hyphal growth (Rogg *et al*., 2012). It is not obvious why dimorphic/polymorphic yeasts such as *C. albicans* have not acquired or evolved MMD-Chs or whether these genes were lost from these lineages. The importance of the Chs from class V in polarized growth has also been stressed since the only Chs present in the reduced genomes of the parasitic Microsporidiomycota belongs to this class (Muszkieta *et al*., 2014).

In fungi, after their synthesis, the Chs are packaged into microvesicles with a diameter of approximately 60 nm, called chitosomes (Leal-Morales *et al.*, 1988). These chitosomes bring the Chs to the hyphal tip of the cell membrane. Recently, it was demonstrated that all the seven Chs from *Neurospora crassa* were contained at the core of the Spitzenkörper (Sánchez-León *et al.*, 2011; Riquelme *et al.*, 2007; Fajardo-Somera

et al., 2015). Chitosomes then fuse with the cell membrane and the chitin synthases get inserted into the interior side of the apical membrane (Riquelme, 2013).

The function of each Chs class differs depending on the fungi and remains poorly characterised at the biochemical level in filamentous fungi (Jiménez-Ortigosa *et al.*, 2012). To date, only the class V Chs of *Wangiella* (*Exophiala*) *dermatitidis* has been isolated by immunoaffinity in an active and soluble form (Abramczyk and Szaniszlo, 2009). Moreover, the phenotypes of the mutants resulting from the deletion of orthologous genes in different fungal species are often very different, which hinders the assessment of each Chs class to a specific function (Jiménez-Ortigosa *et al.*, 2012).

The fact that these MMD-Chs bind actin *in vitro* (Takeshita *et al*., 2005) and that the polar localization of the MMD-Chs in *Aspergillus nidulans*, *Wangiella dermatitidis* and *U. maydis* depends on F-actin (Takeshita *et al.*, 2005; Abramczyk *et al.*, 2009; Treitschke *et al.*, 2010), suggested that these enzymes could be transported on their own along actin to the growth region, at the tip. The function of the motor domain of these proteins has been investigated by Steinberg's group, using the maize pathogen, *U. maydis.* These studies suggest that the MMDs are not required for cytoplasmic motility of chitosomes in cells (Treitschke *et al.*, 2010; Steinberg, 2011). Instead, the MMD of class V Chs supports exocytosis, tethering the vesicle to the cortical actin beneath the plasma membrane, increasing its residence time, thus promoting the subsequent exocytosis and not the retrograde movement back to the cytoplasm (Steinberg, 2011; Schuster *et al.*, 2012).

2. Molecular structure of hybrid MMD-Chs enzymes

Myosins are mechanoenzymes that convert the chemical energy released by ATP hydrolysis into a mechanical force and for that reason are called actin-dependent

molecular motors (Kong *et al*., 2012). These myosins are found in eukaryotic cells and their heavy chains consist of a distinct head (actin binding, ATPase activity and generation of movement), a short neck (that interacts with myosin light chains), and tail domains (that bind the motor). The myosin catalytic head domain contains actin- and ATP-binding sites. Myosins are categorized in several classes (Foth *et al.*, 2006; Mooseker and Foth, 2008; Hartman and Spudich, 2012) and the myosin found in fungal MMD-Chs belongs to class XVII (Taheri-Talesh *et al.*, 2012) which is typified by lacking IQ motifs and so disabled to bind calmodulin-like light chains (Odronitz and Kollmar, 2007) and, in addition to the myosin motor and the chitin synthase domains, contains two specific domains, a cytochrome b_5-like heme/steroid binding domain and a DEK C-terminal domain (Sebé-Pédros *et al*., 2014) (Fig. 2).

No ligands are currently known for the cytochrome b_5-like heme/steroid binding domain, but this might serve as a binding site for lipids and does not seem to bind heme or to be involved in redox reactions (Mifsud and Bateman, 2002). The function of the DEK C-terminal domain also remains unknown (Sheng *et al.*, 2013). In multicellular organisms, DEK is a chromatin associated protein able to modify the structure of DNA and originally described as a proto-oncogene protein (Waldmann *et al.*, 2004; Kappes *et al.*, 2004).

3. Regulation of MMD-Chs genes and protein expression

The fungal cell wall represents the most important structure protecting the cell from deleterious extracellular stimuli and consequently a robust regulation of the enzymes that code for the cell wall components have evolved that are sensitive to environmental perturbations.

The class V and VII Chs genes are usually positioned in a head-to-head configuration (Fig. 3). A head-to-head or bidirectional gene pair configuration is a genomic locus in which two adjacent genes are divergently transcribed from opposite strands of DNA (Trinklein *et al.*, 2004). The region between two transcription start sites is designated as a putative bidirectional promoter and tends to coordinately regulate the transcription of the gene pair (Li *et al.*, 2006). This organization is conserved among their orthologous genes in most of the filamentous fungi whose genome sequences are available (Takeshita *et al.*, 2006; Kim *et al.*, 2009; Larson *et al.*, 2011). A common pattern of transcriptional regulation of both genes was described in *A. nidulans*, in which the levels of *csmB* and *csmA* transcripts respond in a broadly similar fashion to changes in external osmolarities (Takeshita *et al.*, 2006). It is worth noting that *W. dermatitidis* (Abramczyk *et al*., 2009) differs to this pattern and the five *WdCHS* genes exhibit different expression patterns in response to different stimuli (Wang *et al.*, 2002). In *Fusarium oxysporum* and *Aspergillus oryzae*, they are under independent regulation even though these genes have a head-to-head genetic organisation (Martín-Urdíroz *et al*., 2008).

The promoter region of *CSMA* from *A. nidulans* includes a predicted DNA-binding site consensus sequence, CTA(A/T)4 TAG, which is similar to the target sequence of the protein kinase C-mediated MAP kinase pathway that responds to hypo-osmotic stress in *S. cerevisiae* (Heinisch *et al.*, 1999; Dodou and Treisman, 1997; Takeshita *et al.*, 2002). In addition, this promoter region also contains potentially functional promoter elements that would confer the regulation of gene expression in response to stress: 1) STREs (stress-response element), 2) *abaA* response element (ARE) and 3) two HAP (Heme Activator Protein) complex binding sites (Takeshita *et al.*, 2002). These *cis*-acting elements are also found in the upstream regions of *WdCHS5* from *W. dermatitidis*,

(Wang and Szaniszlo, 2000; Liu *et al.*, 2004; Liu and Szaniszlo, 2007). Yet, in *A. fumigatus*, Calcineurin-Dependent Response Elements (CDRE), promoter sequences that bind to the calcineurin pathway transcription factor Crz1p sequences (Spielvogel *et al.*, 2008), are present upstream of the coding region of the class V and VII Chs but also of the Chs of the other classes (Fortwendel *et al.*, 2010). The intergenic region of the genes coding for Chs from class V and VII of *Penicillium digitatum* and *Penicillium chrysogenum* contain a putative binding sequence (TTACTAA) for the transcription factor Yap1p, which is involved in the defence response to oxidative stress (Gandía *et al.*, 2012).

Post-transcriptional regulation of the gene encoding class V MMD-Chs also occurs in *A. nidulans* (Takeshita *et al.*, 2002). Three ORFs are present upstream of the ORF csmA transcript. However, the precise role of each of these has yet to be elucidated (Vilela and McCarthy, 2003). Another regulatory mechanism was identified in CsmA and CsmB from *A. nidulans*, in which a cleavage in a region localized between the myosin motor-like domain and the Chs domain is likely to be responsible for removal of the protein from the respective anchoring regions for subsequent degradation (Takeshita *et al.*, 2002; Takeshita *et al.*, 2006). Similarly, in *W. dermatitidis*, the WdChs5 is also cleaved between the Chs and the MMD during purification procedures, even in the presence of protease inhibitors (Abramczyk and Szaniszlo, 2009). This degradation does not occur *in vivo* in *W. dermatitidis* even after prolonged culture (Liu and Szaniszlo, 2007). In *Colletotrichum graminicola*, cleavage of ChsA was also observed after the *de novo* cell wall synthesis (Amnuaykanjanasin and Epstein, 2006).

In parallel, the mechanisms behind the control of the localization and movement of cell wall-synthesizing enzymes in the hyphae are likely to be another factor of MMD-Chs regulation as explored below.

4. Myosin motor domain structural function

The mechanisms underlying the hyphal growth are still a major challenge in fungal biology. The hypothesis more accepted is that the polar transportation of synthetic enzyme-containing vesicles to the Spitzenkörper is mediated along tracks made of filamentous actin (F-actin) and microtubules in the cytoskeleton (Steinberg *et al*., 2007; Schuster et al., 2012). The Spitzenkörper is located in the cytoplasm of the extreme apex of the hypha, where growth and morphogenesis occur, and contains secretory vesicles and microvesicles, that will fuse with the plasma membrane (Riquelme, 2013). After fusion, chitin synthases, containing several transmembrane domains become inserted in the cell membrane, and initiate synthesis of the cell wall (Munro and Gow, 2001). Besides the importance of the work conducted by the Nobel Prize awarded Randy Schekman (e.g. Chuang and Schekman, 1996; Valdivia *et al*., 2003; Sanchatjate and Schekman, 2006), recent research investigating vesicle trafficking, polarity and hyphal growth (Steinberg, 2011; Riquelme, 2013) has underlined the importance of this process in fungal growth and physiology.

The MMD domain was first recognized in the *CSMA* (Chitin Synthase with Myosin motor-like domain) gene of *A. fumigatus* (Fujiwara *et al.*, 1997). In *A. nidulans*, it was shown that the entire coding region of *CSMA* is translated as a single polypeptide containing both the MMD and the Chs domains (Takeshita *et al.*, 2002). Until recently, little was known about the role of this myosin domain, although some authors have speculated about its involvement in the delivery of the MMD-Chs-attached vesicles along actin filaments to the apical growth region (Fujiwara *et al.*, 1997; Horiuchi *et al.* 1999). In support of this, it was demonstrated that the MMD from *A. nidulans* CsmA and CsmB, a class V Chs and a class VII Chs, respectively, bind F-actin and this binding

activity was required for polar localization and Chs-MMD function (Horiuchi *et al.*, 1999; Takeshita *et al.*, 2005; Takeshita *et al.*, 2006; Tsuizaki *et al.*, 2009). These authors also suggested that MMDs might function as anchors for CsmA and CsmB rather than a motor for transportation to the plasma membrane (Takeshita *et al.*, 2005; Takeshita *et al.*, 2006). Other work on class V Chs also provided evidence that the polar localization of fungal-specific class XVII myosin in *W. dermatitidis* and *U. maydis* is dependent on F-actin (Abramczyk *et al.*, 2009; Treitschke *et al*., 2010). However, in *A. nidulans* and *U. maydis*, this motor domain is not required for class V Chs motility (Treitschke *et al.*, 2010; Takeshita *et al.*, 2005). In fact, in *A. nidulans*, the localization and function of CsmA is dependent on the MMD actin-binding activity, but not on its motor ability (Takeshita *et al.*, 2005). Furthermore, the myosin class XVII is unconventional and has no motile activity (Woolner and Bement, 2009; Schuster *et al.*, 2012). A recent study by Schuster and co-workers has demonstrated bi-directional motility of class V Chs in *U. maydis*. Peripheral actin and myosin-5 mediated transportation of the class V MMD-Chs-bound vesicles to the growth region and lateral cell wall. In parallel, the transport of MMD-Chs-bound vesicles along microtubules is kinesin-1-dependent for anterograde and dynein-dependent for retrograde mobility (Schuster *et al.*, 2012; Steinberg, 2011; Fig. 4). In *U. maydis*, the MMD function of the class V Chs is likely to support apical and lateral secretion by tethering vesicles to the cortical actin on the site of exocytosis (Schuster *et al.*, 2012). This docking leads to an increased residence time near the cell periphery, thus increasing the probability that vesicle fusion with the plasma membrane will take place (Schuster *et al.*, 2012; Steinberg, 2011).

As with class V MMD-Chs it has been proposed that the MMDs of the class VII Chs also function as anchors (Takeshita *et al*., 2006). In *A. nidulans*, the homology between the MMD from CsmA and CsmB is only 21%, whereas the Chs domains share 55%

sequence identity (Takeshita *et al.*, 2006). These data suggest that these MMDs may display different functions (Takeshita *et al.*, 2006). This is underlined by observations that the MMD from CsmA can replace the MMD in CsmB. However, reciprocally the CsmA with MMD from CsmB does not suppress the defects of the Δ*csmA* mutants (Tsuizaki *et al.*, 2013). Furthermore, the MMD of CsmB did not present ATPase or motor activity, but promoted actin binding (Takeshita *et al.*, 2006). Nevertheless, this MMD is also essential for *A. nidulans*, and mutants with a CsmB lacking MMD exhibit defects similar to those of the Δ*csmB* mutant (Tsuizaki *et al.*, 2009).

5. Structural role of MMD-Chs

Almost all mutants disrupted in MMD-Chs genes have morphological aberrations such as balloon-like swellings that in some strains can be suppressed by osmotic stabilizers, indicating that these swellings result from cell wall weakening and that Chs-MMD play a major role in maintenance of hyphal wall integrity. Since these enzymes have also been found in the apex of the hypha and in forming septa (Fajardo-Somera et al., 2015), they are likely to be involved in polarized cell wall synthesis and septum formation. Such mutants also have reduced number or abnormal conidia suggesting they are also essential for conidiogenesis. Table 1 summarizes the common phenotypes of class V Chs and class VII Chs deleted mutants.

In *A. nidulans,* Δ*csmA* and Δ*csmB* mutants display complex morphological alterations, producing hyphal swellings, intrahyphal hyphae and few conidiophores (Horiuchi *et al.*, 1999; Takeshita *et al.*, 2006; Specht *et al.*, 1996). Double deletants of *csmA* and *csmB* appeared to be lethal (Takeshita *et al.*, 2006). The differences observed in the phenotypes of the Δ*csmA* and of the Δ*csmB* (Takeshita *et al.*, 2006; Tsuizaki *et al.*, 2013), indicate that these proteins may have partially specific functions. Deletion of

either *csmA* or *csmB* increased the expression levels of the orthologue (Takeshita *et al.*, 2006).

In *A. fumigatus*, the MMD-Chs enzymes CsmA and CsmB (Class V and class VII) are also essential for hyphal growth and conidium formation. Single and double class V/class VII Chs mutants sporulate poorly, producing fewer and abnormal conidiophores with enlarged vesicles with fewer phialides, and few conidia that have altered pigmentation (Aufauvre-Brown *et al.*, 1997; Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014). These deletant mutants also undergo intrahyphal growth, probably as a mechanism of septal pore closure (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014). Although the deletion of *CSMA* and/or *CSMB* (class V and class VII Chs, respectively) does not affect the overall mycelium chitin content, the re-organization of the cell wall polysaccharides is disturbed, the chitin microfibrils are structurally different and results in increased sensitivity to echinocandins (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014). This suggests that these MMD-Chs are involved in the salvage mechanism that reinforced cell wall chitin, to compensate the deficiency of glucan induced by echinocandins (Fortwendel *et al.*, 2010; Mouyna *et al*., 2010). However, recent work showed that the class III *Chsg* was the only chitin synthase with a major role in recovery from caspofungin exposure (Walker *et al.*, 2015). On the other hand, *Alternaria infectoria* relies on class V and class VII chitin synthases in the salvage mechanism when the fungus is exposed to caspofungin and to nikkomycin Z (Fernandes *et al.*, 2014). As in *Fusarium oxysporum*, *Fusarium verticillioides, Magnaporthe oryzae*, or *Gibberella zeae*, the Chs class V and VII double mutants of *A. fumigatus* are fully viable (Jiménez-Ortigosa *et al.*, 2012). The chitin cell wall content in the double Δ*csmA/csmB* mutants of *A. fumigatus* is similar to wild-type, although this double deletion does not stimulate compensatory expression of Chs from other families. CsmA

and CsmB do not overlap functions and do not compensate for each other (Jiménez-Ortigosa *et al.*, 2012). For example, the class V is involved in conidial chitin synthesis in contrast to the class VII (Jiménez-Ortigosa *et al*., 2012).

In *Neurospora crassa,* the MMD-Chs enzymes play an important role during asexual and sexual reproduction. Δ*chs-V* and Δ*chs-VII* also present a reduced biomass and reduced branching (Fajardo-Somera *et al*., 2015).

In *A. oryzae,* the Chs from class V, encoded by the *csmA* gene, is essential for cell-wall formation during both hyphal growth and conidiation, and the Δ*csmA* mutants has reduced colonial growth rate and conidiation (Müller *et al*., 2002).

From the eight putative chitin synthases found in *C. neoformans,* only the Chs5 has a putative MMD, and *chs5* mutants are not hyper sensitive to 37º C or to cell wall stressors. Nevertheless, this enzyme seems to be involved in the feedback mechanism of transcriptional regulation when other Chs are deleted (Banks *et al*., 2005).

In *Y. lipolytica*, the teleomorph of *Candida lypolitica*, Csm1 and Csm2 (class V and VII Chs enzymes respectively) are involved in the maintenance of cell wall architecture and integrity. The respective mutants were sensitive to cell wall stressors such as Calcofluor White or Congo Red. The other class VII Chs (Csm3) may have overlapping functions with other Chs (Sheng *et al.*, 2013). Mutants of each of these three MMD-Chs enzymes have cell walls with the same chitin content as wild type.

The class V Chs also plays a role in hyphal growth and asexual sporulation and *C. graminicola Cg*Δ*chsV* mutants are unable to form conidia (Werner *et al*., 2007). Also, the class VII Chs is essential for cell wall synthesis of conidia and vegetative hyphae and is localized in the growing tips and septa (Amnuaykanjanasin *et al.*, 2003; Amnuaykanjanasin and Epstein, 2003; Amnuaykanjanasin and Epstein, 2006).

Remarkably, the conidia from these mutants burst during germination in low osmotic media (Epstein *et al.*, 2001).

In *G. zeae*, anamorph *F. graminearum*, the classes V and VII Chs are involved in hyphal growth and septum formation. On the other hand, the conidium production from Δ*GzChs5*, Δ*GzChs7* and Δ*GzChs5/7* double mutants is severely reduced and these mutants fail to produce perithecia (Kim *et al.*, 2009).

6. Relevance of MMD-Chs for infection

Overall, more information is available about the role of MMD-Chs enzymes in vegetative growth and sporulation than during infection (Table 2). However, several reports suggest that the deletion of these enzymes either abolishes phytopathogenicity or dramatically decreases the virulence of the fungus towards the host plant (Table 2).

A number of morphogenetic transitions to form appressoria or lobed hyphopodia are vital for the virulence life styles of plant pathogens and these processes have been shown to frequently be dependent on the ability to synthesise chitin. The class V Chs mutants *C. graminicola* Δ*CgchsV*, are able to form hyphopodia, enabling plant host cell wall penetration but the infecting hyphae exhibit swellings and are not able to proceed with plant colonization; in fact CgChsV proved to be essential for synthesis of rigid appressorial cell walls and consequently, for appressorium-mediated plant infection (Werner *et al.*, 2007). This is also observed in *U. maydis*, that causes smut disease on maize and teosinte, where the classV Chs Mcs1 and Chs6 do not display a critical role in ex planta fungal growth, but are crucial for the initial steps of plant infection. Deleted mutants penetrate plant host cells but then lose growth polarity and form globular aggregates, which are unable to invade deeper plant layers (Weber *et al.*, 2006). Moreover, deleted mutants in the Chs domain are quickly recognized and killed

by the plant, whereas fungi with a deletion of the MMD domain although retaining the ability to invade the host tissue, only elicit a moderate plant defence response (Treitschke *et al*., 2010). UmChs6, a class V Chs, although lacking the MMD domain, is also indispensable for virulence (Garcerá-Teruel *et al*., 2004).

In *M. oryzae,* a hemibiotrophic fungal pathogen that causes rice blast, only the class V[1] Chs is essential for pathogenesis, and Δ*chs6* mutants are non-pathogenic because appressoria formed by these mutants are defective in plant cell penetration (Kong *et al.*, 2012). In *F. verticillioides*, a pathogen of maize, Chs5 (class V Chs) and Chs7 (class VII Chs) are both required for normal hyphal growth and for maximal disease. However, the amount of fumonisin toxin (a major virulence factor of this fungus) is affected in mutants (Larson *et al.*, 2011), questioning the mechanism of the lower pathogenicity. In the citrus postharvest pathogen, *Penicillium digitatum*, PdchsV (class V) and PdchsVII (class VII) are among the genes more induced during infection but not during axenic growth (Gandía *et al.,* 2014). Strains with disruption of the class VII Chs are viable but have a reduction in growth and conidia production. These mutants retain the ability to infect citrus fruit but with lower virulence and do not form visible mycelium and conidia on the fruit (Gandía *et al.*, 2014).

In contrast to the studies above reported, the class V Chs mutant of *B. cinerea* (Δ*BcChs5*) is not more virulent or impaired in cell growth, although this deletion results in an increase of cell wall chitin of 31 % (Cui *et al.*, 2009). On the other hand, BcChs6, a class VII Chs, is required for pathogenicity, hyphal growth of *B. cinerea* and sclerotium formation (Cui *et al.*, 2013). *F. oxysporum* is a multihost pathogen that infects plants and immunocompromised humans. The class V Chs mutants of *F.*

[1] According to the classification adopted in this review and based on the motif present and size of the MMD, we consider the Chs6 from *M. oryzae* is a class V Chs and the Chs5, a class VII Chs. According to the classification adopted in this review and based on the motif present and size of the MMD, we consider the Chs6 from *M. oryzae* is a class V Chs and the Chs5, a class VII Chs.

oxysporum (Δ*chsV*) fail to colonize the vascular system of tomato plants and to invade wounded tomato fruit and these mutants probably due to its increased sensitivity toward hydrogen peroxide (Madrid *et al*., 2003) However, mice injected with microconidia of these mutants, living and heat-killed conidia, died faster than mice infected with the wild type strain, either in immunosuppressed and in immunocompetent mice. The Δ*chsV* mutant killing mechanism was due to respiratory insufficiency because swollen conidia lead to obstruction of the blood flow in the alveolar interstitial capillaries. On the other hand, conidium germination was seen in several organs (Ortoneda *et al.*, 2004). Furthermore, in response to the deletion of one of the Chs-MMD, the expression of the other Chs enzymes was not increased, indicating that there was no compensatory transcriptional mechanisms (Martín-Urdíroz *et al.*, 2008). ChsVb was expressed under low and high osmotic conditions, whilst ChsV was expressed mainly in the absence of osmotic stabilizers (Martín-Urdíroz *et al.*, 2008). The cell walls from all these null mutants have a thicker skeletal inner layer, suggesting a compensatory mechanism of chitin and glucan is activated (Martín-Urdíroz *et al.*, 2008). ChsV is likely to contribute to the structural defence function of the cell wall by preventing the access of antifungal plant compounds (Madrid *et al.*, 2003). The exact mechanism by which MMD-Chs influence the ability of a fungus to infect plants and to elicit an immune response still needs further insights but, besides the importance of melanin deposition in the appressorium, at the penetration peg (Giraldo and Valent, 2013), chitin, by affecting signaling through plasmodesmata affects the spread of a systemic immune response in plants (Faulkner *et al.*, 2013). Given that in most of the phytopathogenic fungi MMD-Chs are essential to infection and that these chitin synthases play an important role in the deposition and organization of chitin at the fungal hyphal tip one can speculate that

the immune response in plant cells may depend on the organization (and not on the amount) of chitin at the appressorium/penetration peg.

The role of the class V has also shown to be essential for virulence of the animal pathogen *W. dermatitidis.* Class V Chs, WdChs5, is required for the sustained growth of *W. dermatitidis* at 37°C and is consequently critical for its virulence in mammals (Liu *et al*., 2004). Mouse survival models of acute infection showed that Δ*wdchs5* mutants were less virulent than wild type (Liu *et al.*, 2004). In *W. dermatitidis*, WdChs5p at 37°C helps in the maintenance of the integrity of the hyphal tips, but does not participate in septation (Abramczyk *et al.*, 2009).

Interestingly, the class V mutants from *A. fumigatus* are virulent, causing pulmonary infection in immunosupressed mice. In fact, the mutants invade the lung tissue and also have a swollen phenotype (Aufauvre-Brown *et al.*, 1997). The surface of wild type *A. fumigatus* conidia is characterised by a mosaic network of hydrophobic nanofibrils, called rodlets, that are composed of hydrophobins, that mask the recognition of immunogenic fungal cell wall components by innate immune cells (Aimanianda *et al.*, 2009). The deletion of *CSMA* gene leads to the disappearance of these layers from the surface in mutant conidia and is associated with potentiated activation of human dendritic cells (Alsteens *et al.*, 2013; Jiménez-Ortigosa *et al.*, 2012). This might explain why the class V mutants from *A. fumigatus* are more virulent (Aufauvre-Brown *et al.*, 1997). In the Δ*csmB* and Δ*csmA*/Δ*csmB* mutants the conidial surface exhibits poorly organized rodlet layers with substantial amounts of exposed mannan and chitin that can engage with pattern recognition receptors of host myeloid cells (Alsteens *et al.*, 2013).

7. Conclusions

Apical growth and chitin synthesis is essential for filamentous fungi to be able to invade and colonize plant and/or animal tissues (Wessels, 1993). Therefore, functional characterization of Chs enzymes is essential not only for the understanding of fungal pathogenicity but also for the identification of novel fungicide targets for agriculture and medicine, since they are absent in animal hosts (Munro and Gow, 1995). Specific inhibitors of fungal Chs such as the polyoxins have been designed and are structural homologues of the Chs substrate, UDP-*N*- acetylglucosamine (Gow and Selitrennikoff, 1984; Muller *et al.*, 1981), but the full potential of chitin as an antifungal target have yet to be realised.

In most of phytopathogenic fungi the deletion of at least one of the class V or for Chs VII genes have morphological growth defects and reduced virulence. The reduced virulence of pathogenic fungi with deleted Chs-MMD genes cannot be attributed to reduced chitin synthesis because in most of these mutants the overall cell wall content is similar to the wild type but rather to the alteration on the chitin microfibrils structure. MMD-Chs deletion is also associated with increased susceptibility to hydrogen peroxide suggesting that these enzymes may be involved directly or indirectly in resistance to host defences mechanisms, possibly due to a decrease in cell wall structural integrity and permeability. In some cases fungi become more virulent in the absence of these MMD-Chs. This might be due to changes in fungal surface structure that leads to higher exposure of inner cell wall layers such as β-glucan that may trigger an immunogenic host response (Alsteens *et al.*, 2013), or because chitin arrangement at the penetration at the hyphal tip is essential to tissue invasion and host immune response (Faulkner et al., 2013). Because these enzymes seem important for fitness and virulence of fungal pathogens they can be regarded as excellent targets for the design of isotype-specific antifungal drugs.

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References

Abramczyk, D., Park, C., Szaniszlo, P.J., 2009. Cytolocalization of the Class V Chitin Synthase in the Yeast, Hyphal and Sclerotic Morphotypes of *Wangiella (Exophiala) dermatitidis*. Fungal Genet. Biol. 46, 28-41.

Abramczyk, D., Szaniszlo, P.J., 2009. Immunoaffinity purification of the class V chitin synthase of *Wangiella (Exophiala) dermatitidis*. Prep. Biochem. Biotechnol. 39, 277-288.

Aimanianda, V., Bayry, J., Bozza, S., Kniemeyer, O., Perruccio, K., Elluru, S.R., Clavaud, C., Paris, S., Brakhage, A.A., Kaveri, S.V., Romani, L., Latgé J.P., 2009. Surface hydrophobin prevents immune recognition of airborne fungal spores. Nature. 460, 1117-1121.

Alsteens, D., Aimanianda, V., Hegde, P., Pire, S., Beau, R., Bayry, J., Latgé, J.P., Dufrêne, Y.F., 2013. Unraveling the nanoscale surface properties of chitin synthase mutants of *Aspergillus fumigatus* and their biological implications. Biophys. J. 105, 320-327.

Amnuaykanjanasin, A., Epstein, L., 2003. A class V chitin synthase gene, *chsA* is essential for conidial and hyphal wall strength in the fungus *Colletotrichum graminicola* (*Glomerella graminicola*). Fungal Genet. Biol. 38, 272-285.

Amnuaykanjanasin, A., Epstein, L., 2006. A class Vb chitin synthase in *Colletotrichum graminicola* is localized in the growing tips of multiple cell types, in nascent septa, and during septum conversion to an end wall after hyphal breakage. Protoplasma. 227, 155-164.

Amnuaykanjanasin, A., Epstein, L., Labavitch, J.M., 2003. Physiological, biochemical, and microscopic analyses of weakened walls of conidia in *Colletotrichum graminicola* with a disrupted class V chitin synthase gene, chsA. Physiol. Mol. Plant. Path. 63, 107–115.

Aufauvre-Brown, A., Mellado, E., Gow, N.A.R., Holden, D.W., 1997. *Aspergillus fumigatus chsE*: A gene related to *CHS3* of *Saccharomyces cerevisiae* and important for hyphal growth and conidiophore development but not pathogenicity. Fungal Genet. Biol. 21, 141-152.

Banks, I.R., Specht, C.A., Donlin, M.J., Gerik, K.J., Levitz, S.M., Lodge, J.K., 2005. A chitin synthase and its regulator protein are critical for chitosan production and growth of the fungal pathogen *Cryptococcus neoformans*. Eukaryot. Cell. 4, 1902-1912.

Bartnicki-Garcia, S., 1968. Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu. Rev. Microbiol. 22, 87–108.

Bowen, A.R., Chen-Wu, J.L., Momany, M., Young, R., Szaniszlo, P.J., Robbins, P.W., 1992. Classification of fungal chitin synthases. Proc. Natl. Acad. Sci. USA. 89, 519-523.

Bulawa, C.E., 1993. Genetics and molecular biology of chitin synthesis in fungi. Annu. Rev. Microbiol. 47, 505-534.

Cheney, R.E., Mooseker, M.S., 1992. Unconventional myosins. Curr. Opin. Cell Biol. 4, 27-35.

Choquer, M., Boccara, M., Gonçalves, I.R., Soulié, M.C., Vidal-Cros, A., 2004. Survey of the *Botrytis cinerea* chitin synthase multigenic family through the analysis of six euascomycetes genomes. Eur. J. Biochem. 271, 2153-2164.

Chuang, J.S., Schekman, R.W., 1996. Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p. J. Cell Biol. 135, 597-610.

Cid, V.J., Duran, A., del Rey, F., Snyder, M.P., Nombela, C., Sánchez, M., 1995. Molecular basis of cell integrity and morphogenesis in *Saccharomyces cerevisiae*. Microbiol. Rev. 59,345-386.

Cui, Z., Ding, Z., Yang, X., Wang, K., Zhu, T., 2009. Gene disruption and characterization of a class V chitin synthase in *Botrytis cinerea*. Can. J. Microbiol. 55, 1267-1274.

Cui, Z., Wang, Y., Lei, N., Wang, K, Zhu, T., 2013. *Botrytis cinerea* chitin synthase BcChsVI is required for normal growth and pathogenicity. Curr. Genet. 59, 119-128.

Din, A.B., Specht, C.A., Robbins, P.W., Yarden, O., 1996. chs-4, a class IV chitin synthase gene from *Neurospora crassa*. Mol. Gen. Genet. 250, 214-222.

Dodou, E., Treisman, R., 1997. The *Saccharomyces cerevisiae* MADSbox transcription factor Rlm1 is a target for the Mpk1 mitogenactivated protein kinase pathway. Mol. Cell. Biol. 17, 1848-1859.

Epstein, L., Bartnicki-Garcia, S., Lippman, E., Bassein, S., Amnuaykanjanasin, A., 2001. Catastrophic wall rupture during conidial germination of a genetically tagged mutant of *Glomerella graminicola*. Mycol. Res. 105, 132-137.

545Fajardo-Somera, R.A., Jöhnk, B., Bayram, Ö., Valerius, O., Braus, G.H., Riquelme, M.,
5462015. Dissecting the function of the different chitin synthases in vegetative growth and
547sexual development in *Neurospora crassa*. Fungal Genet. Biol. 75, 30-45.
548Faulkner, C., Petuschnig, E., Benitz-Alfonso, Y., Beck, M., Robatzek, S., Lipka, V.,
549Maule, A.J., 2013. LYM2-dependent chitin perception limits molecular flux via
550plasmodesmata. Proc. Natl. Acad. Sci. USA. 110, 9166-9170.
551Fernandes, C., Anjos, J., Walker, L.A., Silva, B.M., Cortes, L., Mota, M., Munro, C.A.,
552Gow, N.A., Gonçalves, T., 2014. Modulation of *Alternaria infectoria* cell wall chitin
553and glucan synthesis by cell wall synthase inhibitors. Antimicrob. Agents Chemother.
55458, 2894-2904.
555Fortwendel, J.R., Juvvadi, P.R., Perfect, B.Z., Rogg, L.E., Perfect, J.R., Steinbach, W.J.,
5562010. Transcriptional Regulation of Chitin Synthases by Calcineurin Controls
557Paradoxical Growth of *Aspergillus fumigatus* in Response to Caspofungin. Antimicrob.
558Agents Chemother. 54, 1555-1563.
559Foth, B.J., Goedecke, M.C., Soldati, D., 2006. New insights into myosin evolution and
560classification. Proc. Natl. Acad. Sci. USA. 103, 3681-3686.
561Fu, J., Morris, I.R., Wickes, B.L., 2013. The production of monokaryotic hyphae by
562*Cryptococcus neoformans* can be induced by high temperature arrest of the cell cycle
563and is independent of same-sex mating. PLoS Pathog. 9, e1003335.
564Fujiwara, M., Horiuchi, H., Ohta, A., Takagi M., 1997. A novel fungal gene encoding
565chitin synthase with a myosin motor-like domain. Biochem. Biophys. Res. Commun.
566236, 75–78.
567Gandía, M., Harries, E., Marcos, J.F., 2012. Identification and characterization of chitin
568synthase genes in the postharvest citrus fruit pathogen *Penicillium digitatum*. Fungal
569Biol. 116:654-664.

Gandía, M., Harries, E., Marcos, J.F., 2014. The myosin motor domain-containing chitin synthase PdChsVII is required for development, cell wall integrity and virulence in the citrus postharvest pathogen *Penicillium digitatum*. Fungal Genet. Biol. 67, 58-70.

Garcerá-Teruel, A., Xoconostle-Cazares, B., Rosas-Quijano, R., Ortiz, L., Leon-Ramirez, C., Specht, C.A., Sentandreuc, R., Ruiz-Herrera, J., 2004. Loss of virulence in *Ustilago maydis* by *Umchs6* gene disruption. Res. Microbiol. 155**,** 87-97.

Giraldo, M.C., Valent, B., 2013. Filamentous plant pathogen effectors in action. Nat. Rev. Microbiol. 11, 800-814.

Gow, L.A, Selitrennikoff, C.P., 1984. Chitin Synthetase of *Neurospora crassa*: Inhibition by Nikkomycin, Polyoxin B, and UDP. Curr. Microbiol. 1, 211-216.

Hartman, M.A, Spudich, J.A., 2012. The myosin superfamily at a glance. J. Cell Sci. 125, 1627-1632.

Heinisch, J.J., Lorberg, A., Schmitz, H.P., Jacoby, J.J., 1999. The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in *Saccharomyces cerevisiae.* Mol. Microbiol. 32, 671-680.

Horiuchi, H., Fujiwara, M., Yamashita, S., Ohta, A., Takagi, M., 1999. Proliferation of intrahyphal hyphae caused by disruption of *csmA*, which encodes a class V chitin synthase with a myosin motor-like domain in *Aspergillus nidulans*. J. Bacteriol. 181, 3721-3729.

Jiménez-Ortigosa, C., Aimanianda, V., Muszkieta, L., Mouyna, I., Alsteens, D., Pire, S., Beau, R., Krappmann, S., Beauvais, A., Dufrêne, Y.F., Roncero, C., Latgé, J.P., 2012. Chitin synthases with a myosin motor-like domain control the resistance of *Aspergillus fumigatus* to echinocandins. Antimicrob. Agents Chemother. 56, 6121-6131.

Kappes, F., Scholten, I., Richter, N., Gruss, C., Waldmann, T., 2004. Functional domains of the ubiquitous chromatin protein DEK. Mol. Cell Biol. 24, 6000-6010.

595Kim, J.E., Lee, H.J., Lee, J., Kim, K.W., Yun, S.H., Shim, W.B., Lee, Y.W., 2009.
596*Gibberella zeae* chitin synthase genes, GzCHS5 and GzCHS7, are required for hyphal
597growth, perithecia formation, and pathogenicity. Curr. Genet. 55, 449–459.
598Klis, F.M., 1994. Review: cell wall assembly in yeast. Yeast. 10, 851-869.
599Kong, L.A., Yang, J., Li, G.T., Qi, L.L., Zhang, Y.J., Wang, C.F., Zhao, W.S., Xu, J.R.,
600Peng, Y.L., 2012. Different chitin synthase genes are required for various developmental
601and plant infection processes in the rice blast fungus *Magnaporthe oryzae*. PLoS
602Pathog. 8,e1002526.
603Larson, T.M., Kendra, D.F., Busman, M., Brown, D.W., 2011. *Fusarium verticillioides*
604chitin synthases CHS5 and CHS7 are required for normal growth and pathogenicity.
605Curr. Genet. 57, 177-189.
606Latgé, J.P., 2007. The cell wall: a carbohydrate armour for the fungal cell. Mol.
607Microbiol. 66, 279-290.
608Leal-Morales, C.A., Bracker, C.E., Bartnicki-Garcia, S., 1988. Localization of chitin
609synthetase in cell-free homogenates of *Saccharomyces cerevisiae*: chitosomes and
610plasma membrane. Proc. Natl. Acad. Sci. U.S.A. 85, 8516-8520.
611Lee, K.K., Maccallum, D.M., Jacobsen, M.D., Walker, L.A., Odds, F.C., Gow, N.A.,
612Munro, C.A., 2012. Elevated cell wall chitin in *Candida albicans* confers echinocandin
613resistance *in vivo*. Antimicrob. Agents Chemother. 56, 208-217.
614Lee, S.H., Lee, J., Lee, S., Park, E.H., Kim, K.W., Kim, M.D., Yun, S.H., Lee, Y.W.,
6152009. GzSNF1 is required for normal sexual and asexual development in the
616ascomycete *Gibberella zeae*. Eukaryot. Cell 8, 116–127.
617Lenardon, M.D., Munro, C.A., Gow, N.A., 2010. Chitin synthesis and fungal
618pathogenesis. Curr. Opin. Microbiol. 13, 416-423.

619Li, Y.Y., Yu, H., Guo, Z.M., Guo, T.Q., Tu, K., Li, Y.X., 2006. Systematic analysis of
620head-to-head gene organization: evolutionary conservation and potential biological
621relevance. PLoS Comput Biol. 2, e74.

622Liu, H., Kauffman, S., Becker, J.M., Szaniszlo, P.J., 2004. *Wangiella* (*Exophiala*)
623*dermatitidis* WdChs5p, a class V chitin synthase, is essential for sustained cell growth at
624temperature of infection. Euk. Cell 3, 40-51.

625Liu, H., Szaniszlo, P.J., 2007. Transcription and expression analyses of WdCHS5, which
626encodes a class V chitin synthase with a myosin motor-like domain in *Wangiella*
627*(Exophiala) dermatitidis*. FEMS Microbiol. Lett. 276, 99-105.

628Luque, J.C., Clemons, K.V., Stevens, D.A., 2003. Efficacy of micafungin alone or in
629combination against systemic murine Aspergillosis. Antimicrob. Agents Chemother. 47,
6301452-1455.

631Madrid, M.P., Di Pietro, A., Roncero, M.I., 2003. Class V chitin synthase determines
632pathogenesis in the vascular wilt fungus *Fusarium oxysporum* and mediates resistance
633to plant defence compounds. Mol. Microbiol. 47, 257–266.

634Magellan, H., Drujon, T., Thellend, A., Piffeteau, A., Becker, H.F., 2010. Expression in
635*E. coli* and characterization of the catalytic domain of *Botrytis cinerea* chitin synthase.
636BMC Res. Notes. 3, 299.

637Martín-Udíroz, M., Madrid, M.P., Roncero, M.I., 2004. Role of chitin synthase genes in
638*Fusarium oxysporum.* Microbiology. 150, 3175-3187.

639Martín-Urdíroz, M., Roncero, M.I., González-Reyes, J.A., Ruiz-Roldán, C., 2008.
640ChsVb, a class VII chitin synthase involved in septation, is critical for pathogenicity in
641*Fusarium oxysporum*. Eukaryot. Cell 7, 112-121.

642Mifsud, W., Bateman, A., 2002. Membrane-bound progesterone receptors contain a
643cytochrome b5-like ligand-binding domain. Genome Biol. 3, RESEARCH0068.

644Mooseker, M.S., Foth, B., 2008. The structural and functional diversity of the myosin
645family of actin-based molecular motors. In: Coluccio, L. (Ed), Myosins. Springer, NY,
646pp. 1-34.
647Morcx, S., Kunz, C., Choquer, M., Assie, S., Blondet, E., Simond-Côte, E., Gajek, K.,
648Chapeland-Leclerc, F., Expert, D., Soulie, M.C., 2013. Disruption of *Bcchs4*, *Bcchs6* or
649*Bcchs7* chitin synthase genes in *Botrytis cinerea* and the essential role of class VI chitin
650synthase (*Bcchs6*). Fungal Genet. Biol. 52, 1-8.
651Mouyna, I., Kniemeyer, O., Jank, T., Loussert, C., Mellado, E., Aimanianda, V.,
652Beauvais, A., Wartenberg, D., Sarfati, J., Bayry, J., Prévost, M.C., Brakhage, A.A.,
653Strahl, S., Huerre, M., Latgé, J.P., 2010. Members of protein O-mannosyltransferase
654family in *Aspergillus fumigatus* differentially affect growth, morphogenesis and
655viability. Mol. Microbiol. 76, 1205-1221.
656Müller, H., Furter, R., Zahner, H., Rast, D.M., 1981. Metabolic Products of
657Microorganisms 203. Inhibition of Chitosomal Chitin Synthetase and Growth of Mucor
658rouxii by Nikkomycin Z, Nikkomycin X, and Polyoxin A: A Comparison. Arch.
659Microbiol. 130, 195-197.
660Müller, C., Hjort, C.M., Hansen, K., Nielsen, J., 2002. Altering the expression of two
661chitin synthase genes differentially affects the growth and morphology of *Aspergillus*
662*oryzae*. Microbiol. 148**,** 4025-4033.
663Munro, C.A., Gow, N.A,. 2001. Chitin synthesis in human pathogenic fungi. Med.
664Mycol. 39, 41-53.
665Munro, C.A., Gow, N.A.R., 1995. Chitin biosynthesis as a target for antifungals. In:
666Dixon, G.K., Copping, L.G., Hollomon, D.W. (Eds.), Antifungal Agents: Discovery and
667Mode of Action. Bios Scientific, Oxford, pp. 161-171.

668Muszkieta, L., Aimanianda, V., Mellado, E., Gribaldo, S., Alcàzar-Fuoli, L., Szewczyk,
669E., Prevost, M.C., Latgé, J.P., 2014. Deciphering the role of the chitin synthase families
6701 and 2 in the *in vivo* and *in vitro* growth of *Aspergillus fumigatus* by multiple gene
671targeting deletion. Cell Microbiol. 16, 1784-1805.
672Niño-Vega, G.A., Carrero, L., San-Blas, G., 2004. Isolation of the CHS4 gene of
673*Paracoccidioides brasiliensis* and its accommodation in a new class of chitin synthases.
674Med. Mycol. 42, 51-57.
675Ortoneda, M., Guarro, J., Madrid, M.P., Caracuel, Z., Roncero, M.I.G., Mayayo, E., Di
676Pietro, A., 2004. *Fusarium oxysporum* as a multihost model for the genetic dissection of
677fungal virulence in plants and mammals. Infect. Immun. 72, 1760-1766.
678Riquelme, M., 2013. Tip growth in filamentous fungi: a road trip to the apex. Annu.
679Rev. Microbiol. 67, 587-609.
680Riquelme, M., Bartnicki-García, S., González-Prieto, J.M., Sánchez-León, E., Verdín-
681Ramos, J.A., Beltrán-Aguilar, A., Freitag, M., 2007. Spitzenkörper localization and
682intracellular traffic of green fluorescent protein-labeled CHS-3 and CHS-6 chitin
683synthases in living hyphae of *Neurospora crassa*. Eukaryot. Cell 6, 1853-1864.

684Rogg, L.E., Fortwendel, J.R., Juvvadi, P.R., Steinbach, W.J., 2012. Regulation of
685expression, activity and localization of fungal chitin synthases. Med. Mycol. 50, 2-17.
686Roncero, C., 2002. The genetic complexity of chitin synthesis in fungi. Curr. Genet. 41,
687367-378.
688Ruiz-Herrera, J., Gonzalez-Prieto, J.M., Ruiz-Medrano, R., 2002. Evolution and
689phylogenetic relationships of chitin synthases from yeasts and fungi. FEMS Yeast Res.
6901, 247-256.

Sanchatjate, S., Schekman, R., 2006. Chs5/6 complex: a multiprotein complex that interacts with and conveys chitin synthase III from the trans-Golgi network to the cell surface. Mol. Biol. Cell. 17, 4157-4166.

Sánchez-León, E., Verdín, J., Freitag, M., Roberson, R.W., Bartnicki-Garcia, S., Riquelme, M., 2011. Traffic of chitin synthase 1 (CHS-1) to the Spitzenkörper and developing septa in hyphae of *Neurospora crassa*: actin dependence and evidence of distinct microvesicle populations. Eukaryot. Cell. 10, 683-695.

Schuster, M., Treitschke, S., Kilaru, S., Molloy, J., Harmer, N.J., Steinberg, G., 2012. Myosin-5, kinesin-1 and myosin-17 cooperate in secretion of fungal chitin synthase. EMBO 31, 214-227.

Sheng, W., Yamashita, S., Ohta, A., Horiuchi, H., 2013. Functional differentiation of chitin synthases in *Yarrowia lipolytica*. Biosci. Biotechnol. Biochem. 77, 1275-1281.

Specht, C.A., Liu, Y., Robbins, P. W., Bulawa, C. E., Iartchouk, N., Winter, K. R., Riggle, P. J., Rhodes, J. C., Dodge, C. L., Culp, D. W., Borgia, P. T., 1996. The *chs*D and *chs*E genes of *Aspergillus nidulans* and their roles in chitin synthesis. Fungal Genet. Biol. 20, 153-167.

Spielvogel, A., Findon, H., Arst, H.N., Araújo-Bazán, L., Hernández-Ortíz, P., Stahl, U., Meyer, V., Espeso, EA., 2008. Two zinc finger transcription factors, CrzA and SltA, are involved in cation homeostasis and detoxification in *Aspergillus nidulans*. Biochem. J. 414, 419-429.

Steinberg, G., 2007. Hyphal Growth: a Tale of Motors, Lipids, and the Spitzenkörper. Eukaryot. Cell. 6, 351-360.

Steinberg, G., 2011. Motors in fungal morphogenesis: cooperation versus competition. Curr. Opin. Microbiol. 14, 660-667.

Taheri-Talesh, N., Xiong, Y., Oakley, B.R., 2012. The Functions of Myosin II and Myosin V Homologs in Tip Growth and Septation in *Aspergillus nidulans.* PLoS One. 7, e31218.

Takeshita, N, Ohta, A., Horiuchi, H., 2005. CsmA, a class V chitin synthase with a myosin motor-like domain, is localized through direct interaction with the actin cytoskeleton in *Aspergillus nidulans*. Mol. Biol. Cell. 16, 1961-1970.

Takeshita, N., Ohta, A., Horiuchi, H., 2002. *csmA*, a gene encoding a class V chitin synthase with a myosin motorlike domain of *Aspergillus nidulans*, is translated as a single polypeptide and regulated in response to osmotic conditions. Biochem. Biophys. Res. Commun. 298, 103-109.

Takeshita, N., Yamashita, S., Ohta, A., Horiuchi, H., 2006. *Aspergillus nidulans* class V and VI chitin synthases CsmA and CsmB, each with a myosin motor-like domain, perform compensatory functions that are essential for hyphal tip growth. Mol. Microbiol. 59, 1380-1394.

Trail, F., Common, R., 2000. Perithecial development by *Gibberella zeae*: a light microscopy study. Mycologia 92, 130-138.

Treitschke, S., Doehlemann, G., Schuster, M., Steinberg, G., 2010. The myosin motor domain of fungal chitin synthase V is dispensable for vesicle motility but required for virulence of the maize pathogen *Ustilago maydis*. Plant Cell. 22, 2476-2494.

Trinklein, N.D., Aldred, S.F., Hartman, S.J., Schroeder, D.I., Otillar, R.P., Myers, R.M., 2004. An abundance of bidirectional promoters in the human genome. Genome Res. 14, 62–66.

Tsuizaki, M., Ohta, A., Horiuchi, H., 2013. Myosin motor-like domain of class VI chitin synthase CsmB of *Aspergillus nidulans* is not functionally equivalent to that of class V chitin synthase CsmA. Biosci. Biotechnol. Biochem. 77, 369-374.

Tsuizaki, M., Takeshita, N., Ohta, A., Horiuchi, H., 2009. Myosin motor-like domain of the class VI chitin synthase CsmB is essential to its functions in *Aspergillus nidulans*. Biosci. Biotechnol. Biochem. 73, 1163-1167.

Valdivia, R. H., Schekman, R., 2003. The yeasts Rho1p and Pkc1p regulate the transport of chitin synthase III (Chs3p) from internal stores to the plasma membrane. Proc. Natl. Acad. Sci. USA 100, 10287-10292.

Vilela, C., McCarthy, J.E., 2003. Regulation of fungal gene expression via short open reading frames in the mRNA 5'untranslated region. Mol. Microbiol. 49, 859-867.

Waldmann, T., Scholten, I., Kappes, F., Hu, H.G., Knippers, R., 2004. The DEK protein —an abundant and ubiquitous constituent of mammalian chromatin. Gene 343, 1-9.

Walker, L.A., Lee, K.K., Munro, C.A., Gow, N.A.R., 2015. Caspofungin treatment of *Aspergillus fumigatus* results in ChsG-dependent upregulation of chitin synthesis and the formation of chitin-rich micro-colonies. Antimicrob. Agents Chemother. 59, 5932-5941.

Wang, Q., Liu, H., Szaniszlo, P.J., 2002. Compensatory expression of five chitin synthase genes, a response to stress stimuli, in *Wangiella (Exophiala) dermatitidis*, a melanized fungal pathogen of humans. Microbiol. 148, 2811-2817.

Wang, Z., Szaniszlo, P. J., 2000. *WdCHS3*, a gene that encodes a class III chitin synthase in *Wangiella* (*Exophiala*) *dermatitidis*, is expressed differentially under stress conditions. J. Bacteriol. 182, 874-881.

Weber, I., Assmann, D., Thines, E., Steinberg, G., 2006. Polar localizing class V myosin chitin synthases are essential during early plant infection in the plant pathogenic fungus *Ustilago maydis*. Plant Cell. 18, 225-242.

763Weiss, I.M., Schönitzer, V., Eichner, N., Sumper, M., 2006. The chitin synthase involved
764in marine bivalve mollusk shell formation contains a myosin domain. FEBS Lett. 580,
7651846-1852.

766Werner, S., Sugui, J.A., Steinberg, G., Deising, H.B., 2007. A chitin synthase with a
767myosin-like motor domain is essential for hyphal growth, appressorium differentiation,
768and pathogenicity of the maize anthracnose fungus *Colletotrichum graminicola*. Mol.
769Plant Microbe Interact. 20, 1555-1567.

770Wessels, J.G.H., 1993. Wall growth, protein excretion and morphogenesis in fungi. New
771Phytol. 123, 397-413.

772Woolner, S., Bement, W.M., 2009. Unconventional myosins acting unconventionally.
773Trends Cell Biol. 19, 245-252.

774Zaragoza, O., Rodrigues, M.L., Jesus, M.G., Frases, S., Dadachova, E., Casadevall, A.,
7752009. The capsule of the fungal pathogen *Cryptococcus neoformans.* Adv. Appl.
776Microbiol. 68, 133–216.

777Zujiwara, M., Horiuchi, H., Ohta, A., Takagi, M., 1997. A novel fungal gene encoding
778chitin synthase with a myosin motor-like domain. Biochem. Biophys. Res. Commun.
779236, 75-78.

Figure captions

Fig. 1. Categorization of Chs enzymes and distribution among yeast and filamentous fungi.

Fig. 2. Chitin synthases class V and class VII diagram representing the domains. Chs – chitin synthase domain; MMD – myosin motor domain; Cyt-b5 – cytochrome b5-like heme/steroid domain; DEK_C – DEK C-terminal domain.

Fig. 3. General domain structures of the class V and VII chitin synthases in filamentous fungi and directions and chromosomal positions of the corresponding open reading frames. Chs, chitin synthase domain; Cyt-b5, cytochrome b5-like heme/steroid binding domain; MMD, myosin motor domain; DEK_C, DEK C-terminal domain.

Fig. 4. Transport of class V MMD-Chs-bound vesicles to the growth region and lateral cell wall, in *U. maydis* (Schuster et al., 2012; Steinberg, 2011; Riquelme, 2013). After fusing with the plasma membrane, the enzymes located in the vesicles membrane are inserted in the plasma membrane and participate in the synthesis of the fungal cell wall (Steinberg, 2011).

Table 1 – General morphological alterations and dysfunctions that occur in class V and class Chs deletion mutants.

Fungus	Mutant	Balloon-like structures	Swollen hyphal tips	Intrahyphal occurence	Changes in conidiation	Higher susceptibility to antifungals or cell wall and cell membrane stressors than WT	MMD-Chs localization	Abnormalities in septa formation or distribution	Alteration in chitin contents
Aspergillus fumigatus	Class V Δ*csmA*	✓ (Aufauvre-Brown *et al.*, 1997; Muszkieta *et al.*, 2014)	✓ (Muszkieta *et al.*, 2014)	✓ (Jiménez-Ortigosa *et al.*, 2012 Muszkieta *et al.*, 2014)	Reduced (Aufauvre-Brown *et al.*, 1997; Muszkieta *et al.*, 2014)	✓ (Echinocandins) (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014)			= WT[2] ↓[3] (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014)
	Class VII Δ*csmB*	✓ (Muszkieta *et al.*, 2014)	✓ (Muszkieta *et al.*, 2014)	✓ (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014)	Reduced (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014)				= WT[2,3] ↓[3] (Jiménez-Ortigosa *et al.*, 2012; Muszkieta *et al.*, 2014)
	Δ*csmA*/Δ*csmB*			✓ (Jiménez-Ortigosa *et al.*, 2012)	Reduced (Jiménez-*Ortigosa et al.*, 2012)				= WT[2,3] (Jiménez-Ortigosa *et al.*, 2012)
Aspergillus nidulans	Class V Δ*csmA*	✓ (Specht *et al.*, 1996)	✓[5] (Specht *et al.*, 1996)	✓ (Horiuchi *et al.*, 1999)	Reduced (Horiuchi *et al.*, 1999); Swell and lyse[5] (Specht *et al.*, 1996)		Hyphal apex and septa (Takeshita *et al.*, 2005)	Structurally abnormal and at irregular interval (Horiuchi *et al.*, 1999)	↓[2] (Specht *et al.*, 1996)
	Class VII Δ*csmB*	✓ (Takeshita *et al.*, 2006)	✓[5] (Takeshita *et al.*, 2006)	✓ (Takeshita *et al.*, 2006)	Reduced (Horiuchi *et al.*, 1999);		Hyphal apex and septa (Takeshita *et al.*, 2006)		
	Δ*csmA*/Δ*csmB*	Lethal (Takeshita et al., 2006)							
Aspergillus	Class V	✓ (Müller	✓ (Müller *et al.*,	✓ (Müller	Reduced	✓ (CFW) (Müller			↑[2] (Müller *et al.*,

oryzae	Δ*csmA*	*et al.*, 2002)	2002)	*et al.*, 2002)	(Müller *et al.*, 2002)	*et al.*, 2002)			2002)
Botrytis cinerea	Class V Δ*BcchsV*				No reduced (Cui *et al.*, 2009)	✓ (CFW). Slighly more tolerant to SDS and osmosis regulators (Cui *et al.*, 2009)			↑[2] (Cui *et al.*, 2009)
	Class VII Δ*BcchsV*				Reduced (Morcx *et al.*, 2012)	✓ (CFW, CR, BCIP, SDS) (Cui *et al.*, 2013)		✓ (Morcx *et al.*, 2012)	↑[2] (Cui *et al.*, 2013)
Colletotrichum graminicola	Class V Δ*CgChsV*	✓ (Werner *et al.*, 2007)							
	Class VII T30 (Δ*ChsA*)		✓[1] (Amnuaykanjanasin and Epstein, 2003)		Burst [1] (Epstein *et al.*, 2001)		Hyphal apex (Amnuaykanjanasin and Epstein, 2006)		↓[3] (Amnuaykanjanasin and Epstein, 2003)
Cryptococcus neoformans	Class V Δ*Chs5*					= WT (CR, SDS, caffeine) (Banks *et al.*, 2005)			↓ (Banks *et al.*, 2005)
	Class V w/o MMD *Chs4*Δ					= WT (CR, SDS, caffeine) (Banks *et al.*, 2005)			↓ (Banks *et al.*, 2005)
Fusarium oxysporum	Class V Δ*chsV*	✓ (Madrid *et al.*, 2003)		Not observed (Martín-Urdíroz *et al.*, 2008)	Lemon-like shaped conidia (Madrid *et al.*, 2003)	✓ (plant defence: phytoanticipin, α-tomatine) (Madrid *et al.*, 2003) ✓(CFW, CR, BCIP). Not higher sensitivity to SDS (Martín-Urdíroz *et al.*, 2008)		Not observed (Martín-Urdíroz *et al.*, 2008)	↓[2] (Madrid *et al.*, 2003)
	Class VII Δ*chsVb*	✓ (Martín-Urdíroz *et al.*, 2008)		✓ (Martín-Urdíroz *et al.*, 2008)	Lemon-like shaped conidia (Martín-Urdíroz *et al.*, 2008)	✓ (CFW, CR, BCIP). Not higher sensitivity to SDS (Martín-Urdíroz *et al.*, 2008)		✓ (Martín-Urdíroz *et al.*, 2008)	

	Δ*chsV* / Δ*chsVb*	✓ (Martín-Urdíroz *et al*., 2008)		✓ (Martín-Urdíroz *et al*., 2008)	Lemon-like shaped conidia (Martín-Urdíroz *et al*., 2008)	✓(CFW, CR, BCIP). Not higher sensitivity to SDS (Martín-Urdíroz *et al*., 2008)		✓ (Martín-Urdíroz *et al*., 2008)	
Fusarium verticillioides	Class V Δ*CHS5*	✓ (Larson *et al*., 2011)				✓ (CFW, CR). No more sensitivity to NZ (Larson *et al*., 2011)			↑[2] (Larson *et al*., 2011)
	Class VII Δ*CHS7*	✓ (Larson *et al*., 2011)				✓ (CFW, CR). No more sensitivity to NZ (Larson *et al*., 2011)			↑[2] (Larson *et al*., 2011)
	Δ*CHS5* / Δ*CHS7*	✓ (Larson *et al*., 2011)				✓ (CFW, CR). No more sensitivity to NZ (Larson *et al*., 2011)			↑[2] (Larson *et al*., 2011)
Gibberella zeae	Class V Δ*GzChs5*	✓ (Kim *et al*., 2009)	✓ (Kim *et al*., 2009)	✓ (Kim *et al*., 2009)	Do not produce paricethia (Kim *et al*., 2009)			Woronin bodies (*Kim et al*., 2009)	
	Class VII Δ*GzChs7*	✓ (Kim *et al*., 2009)	✓ (Kim *et al*., 2009)	✓ (Kim *et al*., 2009)	Do not produce paricethia (Kim *et al*., 2009)			Woronin bodies (Kim *et al*., 2009)	
	Δ*GzChs5/7*	✓ (Kim *et al*., 2009)			Do not produce paricethia (Kim *et al*., 2009)				
Magnaporthe oryzae	Class V Δ*chs6*	✓ (Kong *et al*., 2012)	✓ (Kong *et al*., 2012)		Reduced (Kong *et al*., 2012)	✓(CFW, CR, NZ) (Kong *et al*., 2012)			↓[2,3] (Kong *et al*., 2012)
	Class VII Δ*chs5*	Not observed (Kong *et al*., 2012)	Not observed (Kong *et al*., 2012)		Not reduced (Kong *et al*., 2012)	Slightly more resistant to CFW,CR and NZ (Kong *et al*., 2012)			Slight ↑[2,3] (Kong *et al*., 2012)

	Δ*chs5*/Δ*chs6*	✓ (Kong *et al.*, 2012)	✓ (Kong *et al.*, 2012)		Reduced (Kong *et al.*, 2012)	✓(CFW, CR, NZ) (Kong *et al.*, 2012)			
Neurospora crassa	Δ*chs-5*				Reduced (Fajardo-Somera et al., 2015)		Hyphal apex and septa and at interconidial septa (Fajardo-Somera et al., 2015)		Slight ↓[2] (Fajardo-Somera et al., 2015)
	Δ*chs-7*								↓[2] (Fajardo-Somera et al., 2015)
Penicillium digitatum	Class VII Δ*PdchsVII*	✓ (Gandía *et al.*, 2014)			Reduced (Gandía *et al.*, 2014)	✓(CR, CFW, SDS and antifungals as TBZ and IMZ but it antifungal peptides) (Gandía *et al.*, 2014)			↑[2] (Gandía *et al.*, 2014)
Ustilago maydis	Class V w/o MMD Δ*chs6*						Hyphal apex (Weber *et al.*, 2006)		↓[2] (Garcerá-Teruel *et al.*, 2004)
	Class V with MMD Δ*mcs1*	Not observed [4] (Weber *et al.*, 2006)	Not observed (Weber *et al.*, 2006)	Not observed (Weber *et al.*, 2006)			Hyphal apex (Weber *et al.*, 2006)		Slightly x[2] (Weber *et al.*, 2006)
	Δ*mcs1* Δ*chs6*	Yeast more swollen (Weber *et al.*, 2006)	Not observed (Weber *et al.*, 2006)	Not observed (Weber *et al.*, 2006)					
Yarrowia lipolytica	Class V Δ*csm1*					✓(CFW, CR) (Sheng *et al.*, 2013)		Not observed (Sheng *et al.*, 2013)	= WT[2] (Sheng *et al.*, 2013)
	Class VII Δ*csm2*					✓(CFW, CR) (Sheng *et al.*, 2013)			= WT[2] (Sheng *et al.*, 2013)
	Class VII Δ*csm3*								= WT[2] (Sheng *et al.*, 2013)
Wangiella dermatitis	Class V Δ*wdchs5*	Yeast with irregular shape (Liu *et al.*,							

		2004)							

[1] in media with low osmotic pressure

[2] in mycelia

[3] in conidia

[4] Δ*mcs1* without morphological alterations during growh *ex vivo* but during infection, lost of growth polarity and formation of aggregates of spherical cells

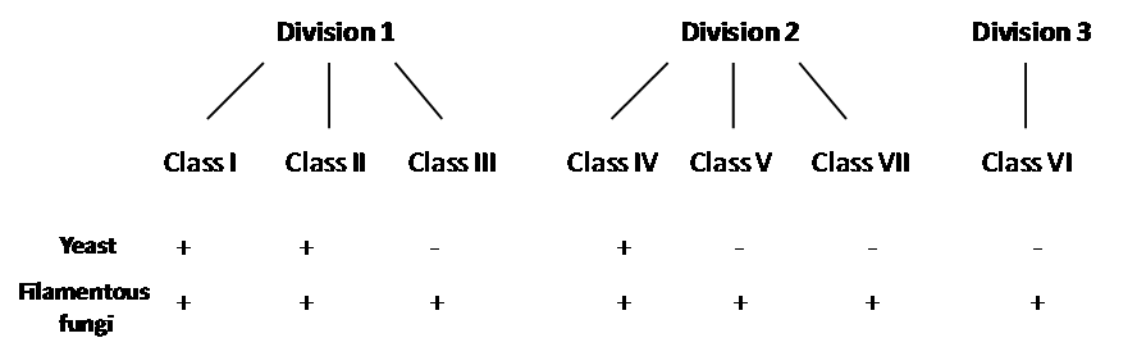
[5] at subapical location

CFW: calcofluor white; CR: congo red; SDS: sodium dodecyl sulfate; BCIP: 5-bromo-4-chloro-3-indolylphosphate; NZ: Nikkomycin Z; ampB: amphotericin B; TBZ: thiabendazole; IMZ: imazalil

Table 2 – MMD- Chs role in infection.

Fungus	Virulence (plant and animal host)	Penetration of the host cell and tissue invasion	Sensitivity to H_2O_2	Growth at 37ºC
Aspergillus fumigatus	ChsV mutants are virulent in mice and form swollen hypha in the lungs (Aufauvre-Brown *et al.,*, 1997)			
Botrytis cinerea	Δ*Bcchs5* are equally virulent (Cui *et al.*, 2009); the class VII Chs is essential to virulence (Cui *et al.*, 2013)			
Colletotrichum graminicola	Δ*CgChsV* is non pathogenic (Werner *et al.*, 2007)	Penetration of the plant cell; hyphal swelling prevents infection progression (Werner *et al.*,2007)	Δ*CgChsV* similar sensitivity to WT (Werner *et al.*, 2007)	
Cryptococcus neoformans				*chs5*Δ retain ability to grow at 37ºC (Banks *et al.*, 2005)
Fusarium oxysporum	Δ*chsV,* Δ*chsVb* and double Δ*chsV* Δ*chsVb* are non-pathogenic for plants; Δ*chsV* mutants cause higher mortality in mice (Ortoneda *et al.*, 2004; Martín-Urdiroz *et al.*, 2008)	Mutants penetrate but fail to colonize tomato plant and invade tomato fruits (Ortoneda *et al.*, 2004; Martín-Urdiroz *et al.*, 2008). Δ*chsVb* macerate the fruit tissue (Martín-Urdíroz *et al.*, 2008).	Higher sensitivity to H_2O_2 (Madrid *et al.*, 2003)	
Fusarium verticillioides	Δ*CHS5*, Δ*CHS7,* Δ*CHS5*/Δ*CHS7* have lower infection rate (Larson *et al.*, 2008)		Δ*CHS5*, Δ*CHS7,* Δ*CHS5*/Δ*CHS7* mutants are more sensitive to H_2O_2 (Larson *et al.*, 2011)	
Gibberella zeae	Δ*GzCHS5* and Δ*GzCHS7* are avirulent (Kim *et al.*, 2009)			
Magnaporthe oryzae	chs6 mutant (class V) non-pathogenic to plants. chs5 mutant (class VII) pathogenic(Kong *et al.*, 2012)	*chs6* mutant and *chs5 chs6* double mutant fail to penetrate the plant (Kong *et al.*, 2012)	*chs5* and *chs6* mutants less sensitive *chs5 chs6* double mutants more sensitive (Kong *et al.*, 2012)	
Penicillium digitatum	*PdchsVII* mutants havelower virulence (Gandía *et al.*, 2014)	Ability to infect citrus fruit without formation of mycelia and conidia in the fruit (Gandía *et al.*, 2014).	*PdchsVII* mutants more sensitive to H_2O_2 (Gardia *et al.*, 2014)	
Ustilago maydis	Δ*chs6* and Δ*mcs1*	Δ*mcs1* penetrate the	Δ*mcs1* similar	

	lose virulence (Garcerá-Teruel *et al.*, 2004; Weber *et al.,* 2006)	plant epidermic cells but then loses growth polarity and swelling prevents invasion (Weber *et al.*, 2006). Chs domain is determinant to evade plant detection and defence mechanisms (Treitschke *et al.*, 2010)	sensitivity to WT (Weber *et al.*, 2006)	
Wangiella dermatitis	Lower virulence in mice (Liu *et al.*, 2004)			No growth at 37ºC (Liu *et al.*, 2004)


Division 1
Division 2
Division 3
Class I
Class II
Class III
Class IV
Class V
Class VII
Class VI
Yeast
+
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Filamentous fungi
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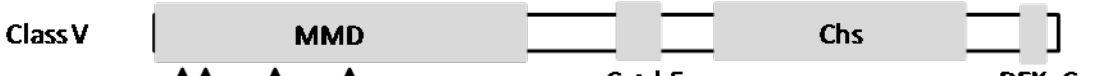
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Chs
ATP
binding site
Switch I
Switch II
Cyt-b5
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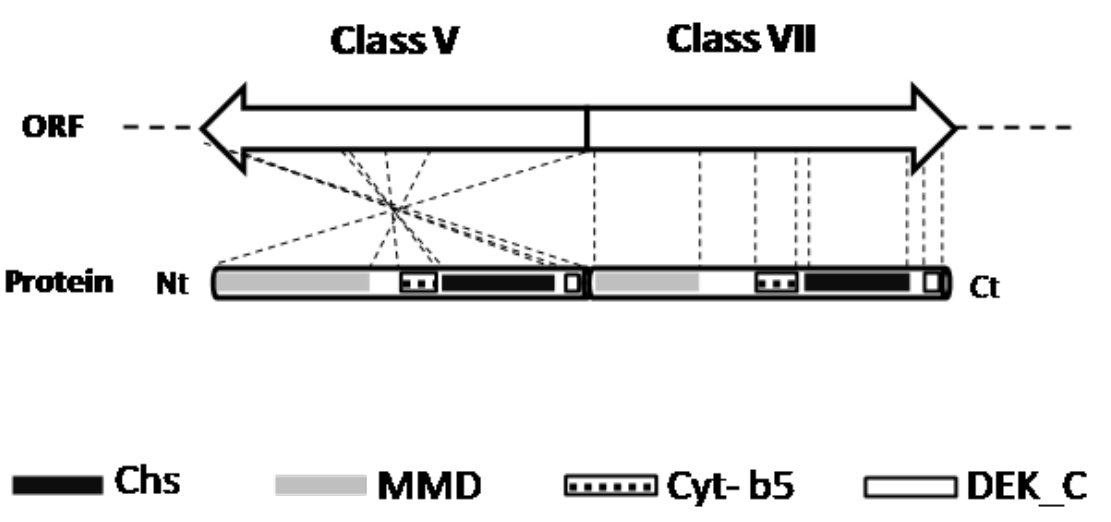
Class VII
MMD
Chs
Cyt-b5
DEK_C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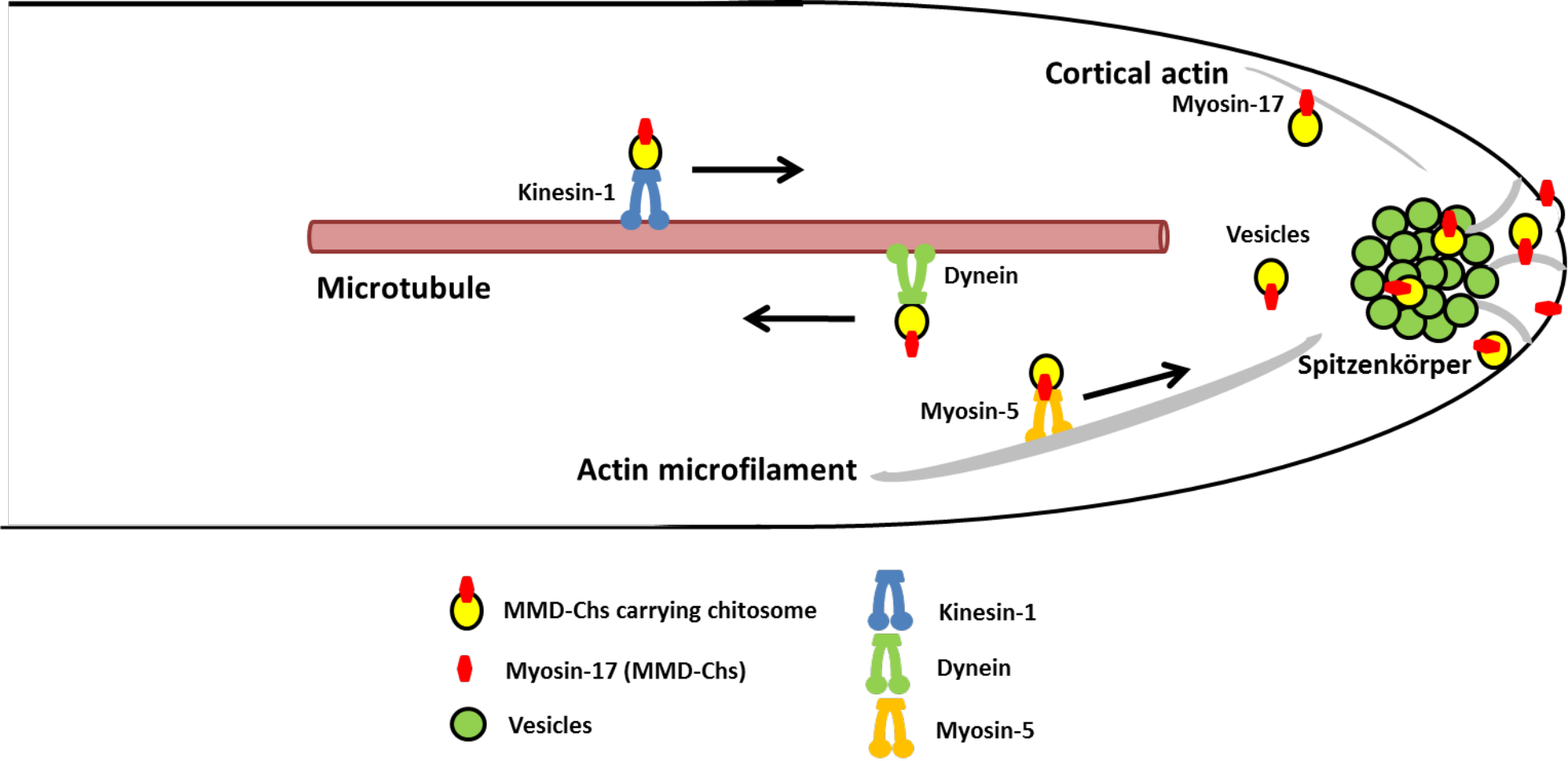
Cortical actin
Myosin-17
Kinesin-1
Vesicles
Microtubule
Dynein
Spitzenkörper
Myosin-5
Actin microfilament
MMD-Chs carrying chitosome
Myosin-17 (MMD-Chs)
Vesicles
Kinesin-1
Dynein
Myosin-5