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REVIEW

Fungal chitinases: function, regulation, and potential roles in plant/pathogen interactions

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Abstract In the past decades our knowledge about fungal cell wall architecture increased tremendously and led to the identification of many enzymes involved in polysaccharide synthesis and remodeling, which are also of biotechnological interest. Fungal cell walls play an important role in conferring mechanic stability during cell division and polar growth. Additionally, in phytopathogenic fungi the cell wall is the first structure that gets into intimate contact with the host plant. A major constituent of fungal cell walls is chitin, a homopolymer of *N*-acetylglucosamine units. To ensure plasticity, polymeric chitin needs continuous remodeling which is maintained by chitinolytic enzymes, including lytic polysaccharide monooxygenases *N*-acetylglucosaminidases, and chitinases. Depending on the species and lifestyle of fungi, there is great variation in the number of encoded chitinases and their function. Chitinases can have housekeeping function in plasticizing the cell wall or can act more specifically during cell separation, nutritional chitin acquisition, or competitive interaction with other fungi. Although chitinase research made huge progress in the last decades, our knowledge about their role in phytopathogenic fungi is still scarce. Recent findings in the dimorphic basidiomycete *Ustilago maydis* show that chitinases play different physiological functions throughout the life cycle and raise questions about their role during plant-fungus interactions. In this work we summarize these functions, mechanisms of chitinase regulation and their putative role during pathogen/host interactions.

Keywords Fungal chitinases · Plant/fungus interaction · Ustilago maydis · Cytokinesis · Effector proteins · Immunity

Introduction

The cell wall is the outermost part of fungal cells which is in constant contact with the environment. It primarily consists of polysaccharides (mainly glucans and chitin), which account for ~90 % of the walls dry weight (Latgé 2007). Both rigidity and plasticity of the cell wall are essential in maintaining normal growth and survival of the fungus. In recent years, cell wall-remodeling enzymes attracted the interest of basic and applied researchers as their role in biotechnological applications, e.g., biofuel production from plant or fungal biomass broadens (Eijsink et al. 2008).

Chitin is the most abundant amino polysaccharide and after cellulose the second most abundant biopolymer in the biosphere (Hours and Gortari 2013). It is composed of β-1-4 linked *N*-acetylglucosamine (GlcNAc) residues forming a linear homopolymer. As an essential scaffold material chitin is found in the exoskeleton of Ecdysozoa and in fungal cell walls (Bowman and Free 2006; Merzendorfer 2003), where it provides mechanic stability. Chitin remodeling is carried out by an interplay of membrane-associated chitin synthases (CHS; EC 2.4.1.16), which catalyze the de novo synthesis of chitin from UDP-GlcNAc (Roncero 2002; Lenardon et al. 2010), and chitin hydrolyzing enzymes (Latgé 2007). Chitinolytic enzymes include lytic polysaccharide monooxygensases (LPMOs) of the auxiliary activity 10 family (AA10; EC N/A),

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N-acetyl-glucosaminidases (EC 3.2.1.52), and chitinases (EC 3.2.1.14; for a classification of carbohydrate active enzymes, see www.cazy.org; Lombard et al. 2014).

Fungal chitinases exclusively belong to the glycosyl hydrolase (GH) 18 family, which is further sub-classified in three groups and five classes (I–V) according to their domain architecture and sequence homology (Henrissat 1991; Patil et al. 2000). Although GH18 chitinases share a common catalytic mechanism, they can either have endo- or exo-acting character, i.e., they hydrolyze at random positions in the polymer or degrade it from one terminus, respectively (Horn et al. 2006).

The number of chitinases encoded in fungal genomes is highly variable and ranges from only one GH18 family member in the yeast *Schizosaccharomyces pombe* to more than 30 in mycoparasitic *Trichoderma* spp. (Gruber and Seidl-Seiboth 2011). Most of our current knowledge about the physiological function of fungal chitinases has been gained from studies on model species of the phylum Ascomycota, e.g., *Saccharomyces cerevisiae, Candida albicans, Neurospora crassa, Ashbya gossipii*, and *Trichoderma harzianum* (Kuranda and Robbins 1991; Dünkler et al. 2005; Tzelepis et al. 2012; Dünkler et al. 2008; Carsolio et al. 1994), but insights into their role in Basidiomycota are still scarce. In this review we summarize the physiological roles of fungal chitinases and highlight recent findings in *Ustilago maydis* as a representative of plant pathogenic basidiomycetes. We also address questions that go beyond the current knowledge and concern possible roles of fungal chitinases in plant/fungus interactions.

Chitin synthesis

To maintain plasticity, fungal cell walls need continuous remodeling, which involves synthesis, cross-linking, and degradation of cell wall polysaccharides (reviewed in Teparić and Mrša 2013). The enzymatic repertoire which maintains these processes is conserved across the fungal kingdom (reviewed in Ruiz-Herrera et al. 2002). Additionally, a regulatory pathway for cell wall maintenance in response to stress, the cell wall integrity pathway, is well-described in *S. cerevisiae* (reviewed in Levin 2011).

De novo chitin synthesis is catalyzed by membrane-associated chitin synthases (reviewed in Roncero 2002 and Lenardon et al. 2010). The number of chitin synthase genes in fungi ranges from only one member in *S. pombe* to eight in the filamentous ascomycetes *Aspergillus fumigatus* and *A. nidulans* or the basidiomycetes *Cryptococcus neoformans* and *U. maydis* (Lenardon et al. 2010).

Several chitin synthases have already been functionally characterized. They act in various processes such as septum formation, cell division, polar growth, stress response, or pathogenic development (Ichinomiya et al. 2005; Silverman et al. 1988; Kim et al. 2009; Weber et al. 2006; Treitschke et al. 2010; Cui et al. 2013). Interestingly, many chitin synthases are of zymogenic nature and localize to the plasma membrane at sites of polar growth where they are further processed into their active form (Starr et al. 2012; Valdivia and Schekman 2003; Bowen et al. 1992; Durán et al. 1975). The delivery to these sites involves the microtubule and actin cytoskeleton, motor proteins like kinesin and myosin, secretory vesicles or so called chitosomes, and in some cases an N-terminal myosin-like motor domain (Sietsma et al. 1996; Takeshita et al. 2005; Schuster et al. 2012; Egan et al. 2012). Once associated with the plasma membrane, chitin synthases catalyze the vectorial synthesis of the chitin chains from activated UDP-GlcNAc monomers, releasing the nascent polymer to the extracellular space. There, single chitin chains associate with each other through intramolecular interactions forming chitin microfibrils and covalent crosslinking to cell wall glucans occurs (Cabib and Arroyo 2013; Cabib et al. 2007; Teparić and Mrša 2013). This crosslinking is maintained by transglycosylases or by intrinsic transglycosylation activity of chitinases, as described for Chit42 and Chit33 from *T. harzianum* or ChiB1 from *A. fumigatus* as well as for bacterial chitinases (Zakariassen et al. 2011; Hartl et al. 2012).

The hierarchy of chitin degradation

The breakdown of polymeric chitin in the fungal cell wall is mediated by a number of chitinolytic enzymes, including LPMOs, chitinases, and *N*-acetylglucosaminidases, which work in concert for efficient, but tightly regulated degradation (Fig. 1). LPMOs and endo-acting GH18 chitinases insert strand breaks at random positions within the chitin chains. The exact mechanism how LPMOs insert strand breaks is not understood, but accumulating evidence shows that it involves binding of a copper ion by the imidazole and the peptide-backbone amino group of a conserved N-terminal histidine and the imidazole of a further downstream histidine residue (Nakagawa et al. 2015; Vaaje-Kolstad et al. 2005). The catalytic mechanism also involves electron donors such as ascorbic acid or reduced glutathione (Vaaje-Kolstad et al. 2010). In the current model, the copper ion enables transport of an electron to molecular oxygen, thereby creating a superoxo-intermediate which then initiates cleavage of the polymer. By contrast, the catalytic mechanism of chitinases is well-characterized. To insert strand breaks, GH18 chitinases employ a neighboring group participation mechanism, in which the *N*-acetamido carbonyl oxygen serves as nucleophile (Terwisscha van Scheltinga et al. 1995). A conserved glutamate

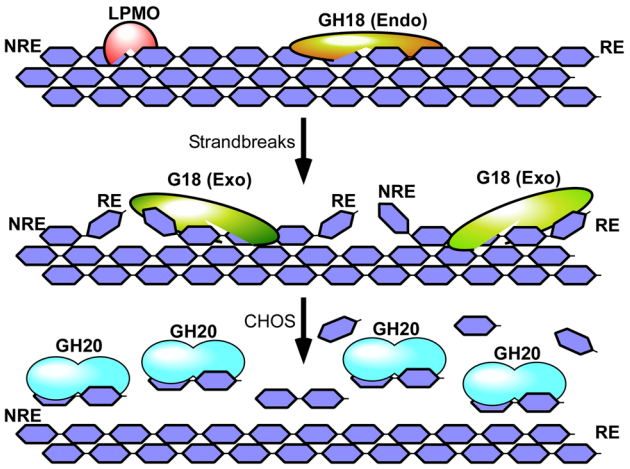

Fig. 1 The hierarchical machinery of chitin degradation. In fungal cell walls polymeric chitin (*purple hexagons*) is densely packed into higher ordered microfibrils. Lytic polysaccharide monooxygenases (LPMOs) and endo-acting GH18 chitinases insert strand breaks at random positions within the polymer, thereby generating reducing (RE) and non-reducing ends (NRE) and loosening up the packed substrate. LPMOs use an oxidative mechanism, while GH18 chitinases use a neighboring group participation mechanism. Exo-acting GH18 chitinases subsequently cleave chito-oligosaccharides (CHOS), predominantly $(GlcNAc)_2$, from either the reducing or the non-reducing ends also using a neighboring group participation mechanism. These CHOS serve as substrate for *N*-acetylglucosaminidases of the GH20 family which cleave GlcNAc monomers from the non-reducing end

in the catalytic center (DxxDxDxE) serves as general acid/base and protonates the leaving group, thereby creating an oxazolium intermediate which, after break down, leads to hydrolysis of the polymer (van Aalten et al. 2001). During hydrolysis the anomeric configuration of the substrate is retained.

Cleavage of polymeric chitin by LMPOs or endo-acting chitinases generates free reducing and non-reducing ends. Such free ends serve as targets for processive GH18 chitinases, which employ the same catalytic mechanism as endo-acting chitinases (Vaaje-Kolstad et al. 2010, 2012). Notably, the increased processivity depends on the architecture of the binding cleft, which is "tunnel-shaped", as opposed to a shallow and rather "open" architecture in non-processive, endo-acting chitinases (Horn et al. 2006; Vaaje-Kolstad et al. 2013). Upon cleavage, processive chitinases release short chito-oligosaccharides (CHOS), mainly $(GlcNAc)_2$ and $(GlcNAc)_3$, from either the reducing or non-reducing end without dissociating from the substrate, leading to the complete decomposition of the strand (Horn et al. 2006; Zakariassen et al. 2009).

CHOS, mainly $(GlcNAc)_2$, are the preferred substrate for *N*-acetylglucosaminidases of the GH20 family which catalyze the further degradation to GlcNAc from the non-reducing end (Langner et al. 2015; Chen et al. 2015). Similar to chitinases they use a substrate assisted, retaining mechanism (Drouillard et al. 1997; Jones and Kosman 1980) in which a conserved glutamate of the catalytic diad (D–E) serves as general acid/base. After protonation the aspartate might stabilize the oxazolium intermediate during hydrolysis (Tews et al. 1996; Williams et al. 2002).

Remarkably, the chitinolytic machinery shares similarities with the enzymatic repertoire which is used for the breakdown of other complex biopolymers such as cellulose (Beeson et al. 2015; Igarashi et al. 2011; Phillips et al. 2011). Therefore mechanistic insights into the mode of action, localization, and regulation of chitinolytic enzymes might also contribute to the understanding of these other enzymatic systems. In the future, this knowledge may even be applied to drive forward industrial applications, like, e.g., the efficient degradation of plant and fungal biomass for biofuel production (Eijsink et al. 2008).

Physiological functions of fungal chitinases

The biological functions of chitinases are very diverse and include roles in yeast and filament morphogenesis, autolysis, acquisition of chitin for nutritional purposes, and mycoparasitism (Kuranda and Robbins 1991; Shin et al. 2009; Yamazaki et al. 2007; Leake and Read 1990; Gruber et al. 2011). In general, the number of chitinase genes is lower in yeasts as compared to filamentous fungi. *S. pombe* possesses just one, *S. cerevisiae* only two chitinases (Karlsson and Stenlid 2008). By contrast, filamentous fungi can contain expanded chitinase gene families. The filamentous ascomycetes *A. nidulans* and *A. fumigatus* have 19 and 20 genes encoding GH18 enzymes, respectively, and huge gene families are described for mycoparasitic species like *T. virens* with 36 chitinase genes (Gruber and Seidl-Seiboth 2011). Due to a high degree of functional redundancy within the GH18 family, a major challenge in chitinase research is to assign specific physiological functions to single members of this family. Often it is necessary to investigate multiple deletion mutants to observe phenotypic alterations. Notably, dimorphic fungi, like the ascomycete *C. albicans* (four chitinases; Dünkler et al. 2005) or the plant pathogenic basidiomycete *U. maydis* (three chitinases; Kämper et al. 2006; Koepke et al. 2011) often encode rather small numbers of chitinase genes which facilitates functional characterization during both, yeast-like and filamentous growth.

The role of chitinases during yeast-like growth

First knowledge about the biological function of chitinases was gained in early studies in the model yeast *S. cerevisiae*. The chitinase *Sc*Cts1p is essential for chitin degradation during cell division and deletion of the *CTS1* gene leads

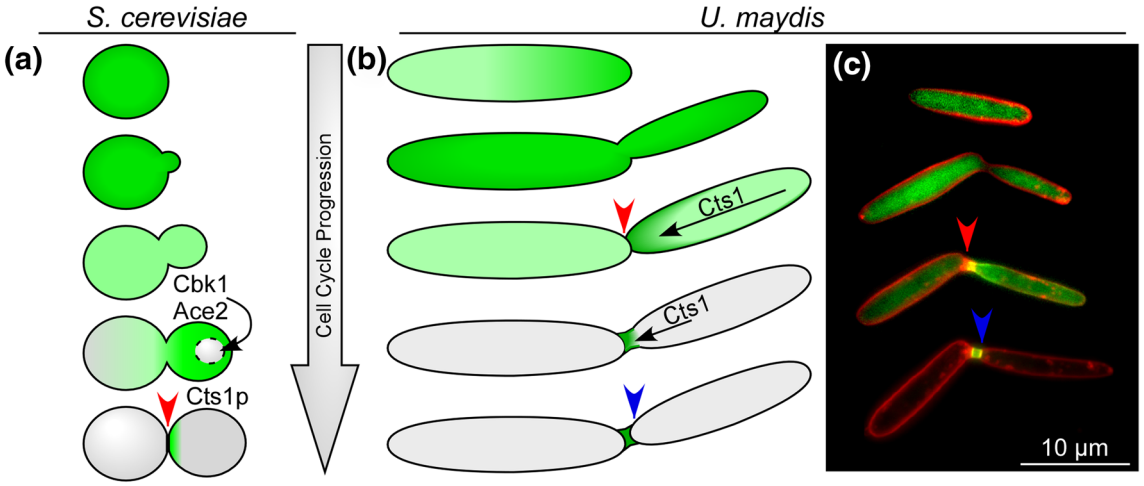

Fig. 2 Spatio-temporal regulation of chitinases in *S. cerevisiae* and *U. maydis*. **a** In *S. cerevisiae* CTS1 is expressed in the mother and the budding daughter cell during early stages of cell division. As the daughter cell continues budding *CTS1* expression becomes downregulated. In late mitotic stages *CTS1* expression is restricted to daughter cells by the concerted action of *Sc*Cbk1p and *Sc*Ace2p, both constituents of the RAM network. The *Sc*Cts1p protein eventually localizes to the daughter cell site of the primary septum (*red arrowhead*) facilitating cell separation. **b** In *U. maydis*, the chitinase *Um*Cts1 is equally distributed in both, the mother and daughter cell early in cytokinesis. After insertion of the primary septum (*red arrowhead*) by the mother cell *Um*Cts1 dynamically localize to the division zone between mother and daughter cell. This relocalization is exclusively maintained by the daughter cell. Upon insertion of the secondary septum (*blue arrowhead*) by the daughter cell, *Um*Cts1 accumulates in the fully established division zone to degrade remnant chitin and facilitate cell separation. **c** Co-localization of *Um*Cts1:eGfp (*green*) and cell membrane stained with the lipophilic dye FM4-64 (*red*) in dividing *U. maydis* cells. After accumulation of membrane material on the mother side of the division zone (*red arrowhead*) *Um*Cts1 relocalization starts within the daughter cell. By the insertion of the secondary septum and the daughter cell membrane (*blue arrowhead*) Cts1 is delimited within the division zone

to a defect in cell separation and the formation of multicellular aggregates (Kuranda and Robbins 1991). During cell cycle *Sc*Cts1p dynamically localizes to the mother-daughter neck, where it contributes to the degradation of the primary septum (Fig. 2a; Colman-Lerner et al. 2001). Similarly, the *Sc*Cts1p homolog of the yeast *Kluyveromyces lactis* (Colussi et al. 2005) and *Ca*Cht3p of the opportunistic pathogen *C. albicans* are required for cytokinesis. In complementation studies Dünkler et al. (2005) showed, that *Ca*Cht3p indeed is the functional homolog of *Sc*Cts1p. Similar phenotypic observations were made in *S. pombe*. However, here the primary septum is mainly composed of glucan, which is degraded by the glucanases *Sp*Agn1 and *Sp*Eng1 (Martin-Cuadrado 2003; García et al. 2005). Consequently, deletion of the glucanase genes leads to defects in cell separation, reminiscent of chitinase deletions in other yeasts. By contrast, the deletion of single chitinase genes in the dimorphic smut *U. maydis* had no effect on yeast cell morphogenesis or cell separation (Koepke et al. 2011). Only double deletions of the chitinase genes *cts1* and *cts2* cause severe defects in cell separation suggesting redundant functions in this growth stage. Both chitinases contribute to the degradation of remnant chitin in an extracellular division zone between the primary and secondary septum, whereas septum formation remains unaffected (Langner et al. 2015). Notably, leaderless *Um*Cts1 dynamically localizes to the fragmentation zone in a cell cycle dependent, daughter cell-specific manner during late stages of cell division (Fig. 2b, c), while *Um*Cts2 contains a signal peptide for secretion (Langner et al. 2015). Intriguingly, these results indicate that functional redundancy even exists between *Um*Cts1 and *Um*Cts2, two chitinases which employ different secretory mechanisms.

During sporulation, the second *S. cerevisiae* chitinase, *Sc*Cts2p, is involved in asci formation (Giaever et al. 2002). Similarly, the chitinase *Ag*Cts2 in *A. gossypii* plays an important role in spore formation (Dünkler et al. 2005, 2008). The sporulation defect caused by the deletion of *cts2* can be complemented by inserting *CTS2* from *S. cerevisiae* or *CHT4* from *C. albicans*, indicating a comparable function in the sporulation process (Dünkler et al. 2008). By contrast, in *U. maydis* teliospore formation is independent of chitinase activity, since quadruple mutants lacking all chitinase activity are able to produce pigmented teliospores (Langner et al. 2015).

A different, cell cycle unrelated function of chitinases has been reported in the opportunistic basidiomycete *C. neoformans*. Deletion mutants of the whole GH18 gene family comprising four members revealed none of the above described phenotypes. Instead, the mutants display reduced tolerance against the cell wall stress inducing agent SDS and defects in mating suggesting a role of these

chitinases in cell wall maintenance during yeast-like growth (Baker et al. 2009). In *C. albicans* cell wall stress resistance is enhanced during biofilm formation. An extracellular matrix composed of polysaccharides and *N*-acetylglucosamine is produced to protect the cells against antimicrobial compounds (Mathé and van Dijck 2013). It is conceivable that chitinases might also be involved in the conversion of chitin to contribute sugars to this matrix. However, the role of chitinases in tolerance against cell wall stress seems not to be conserved in other model systems. In line with this, a comprehensive genetic analysis in *U. maydis* could not reveal a function of chitinases under various cell wall stress inducing conditions (Langner et al. 2015).

Taken together, chitinases play diverse, but important roles in cell wall remodeling during different stages of the fungal life cycle influencing cell wall integrity, cell separation, mating, and stress resistance. However, the physiological relevance of these functions remains to be elucidated.

The role of chitinases during filamentous growth

Functional characterization of chitinases in filamentous fungi is often difficult because of expanded gene families and functional redundancy. Nevertheless, a number of studies addressed this issue and functions of chitinases in hyphal growth, autolysis, and mycoparasitism were described (reviewed in Hartl et al. 2012; Adams 2004). During hyphal growth extension of the filament is supposed to involve controlled cell wall remodeling. Consistently, in *N. crassa* the deletion of the chitinase gene *chit-1* leads to a reduction in hyphal growth suggesting that cell wall-associated *Ng*Chit-1 has a plasticizing function (Tzelepis et al. 2012; Maddi et al. 2009). Although in *A. nidulans An*ChiA localizes to polar growth sites of germ tubes and filaments, pointing at an involvement in polar growth, no function in filamentous growth could be observed in the deletion mutant, likely due to redundancy within the chitinase family (Yamazaki et al. 2008). By contrast, *ChiB* single deletions mutants have drastically reduced chitinolytic activity and show enhanced aggregation in the stationary phase, indicating a role during autolytic degradation of the cell wall (Yamazaki et al. 2007).

In the phytopathogenic basidiomycete *U. maydis*, mating of compatible partners on the plant surface induces filamentous growth (Feldbrügge et al. 2004). The filament represents the virulent form which is able to form appressoria and penetrate its host plant *Zea mays*. During this stage of the life cycle septa are inserted at the basal pole of the filament leading to the formation of empty sections. Interestingly, deletion mutants of *cts1* tend to aggregate at their basal poles leading to fast sedimentation in liquid culture, which indicates a role in autolytic chitin degradation at the empty sections of filaments (Koepke et al. 2011). This assumption was substantiated by co-localization studies revealing specific localization patterns of *Um*Cts1 at the cell wall and at septa of empty sections (Langner et al. 2015). Despite that basal localization, *Um*Cts1 shows a gradual increase towards the growing pole which suggests additional functions in polar growth, but deletion mutants did not show altered filament formation. Noteworthy, *Um*Cts2 which acts redundantly in yeast cells does not contribute to the overall chitinolytic potential in filaments, suggesting specific downregulation of this chitinase at the passage from saprotrophic to biotrophic growth (Langner et al. 2015).

In sum, in filamentous growing fungi chitinases seem to have only minor effects on the establishment of unipolar growth, possible due to an intimate regulation of chitin synthesis and degradation. Nevertheless, chitinases are important in autolytic processes that might facilitate growth under non-optimal conditions, such as starvation. Furthermore, chitinases might also be involved in the defense against competing fungi, and thus could contribute to overall fitness in complex environments such as soil or plant microbiomes.

Regulation of chitinases

Depending on their physiological function fungal chitinases differ in their regulation. Housekeeping chitinases are thought to be involved in continuous remodeling of the cell wall and are therefore constitutively expressed. By contrast, specialized chitinases which function in degrading exogenous chitin or during specific growth processes, e.g., cell division/separation or autolysis, require spatio-temporal regulation of chitinase activity.

A comprehensive gene expression analysis of twelve GH18 chitinases in *N. crassa* showed selective induction under varying conditions, while phenotypes were only detected in two of the single deletion mutants (Tzelepis et al. 2012). *gh18-10* is constitutively expressed under the tested growth conditions and its deletion results in reduced growth as expected for a housekeeping chitinase. Similarly, the chitinase genes *gh18-1* and *gh18-2* are constitutively expressed albeit at much lower level, also indicating general functions in cell wall remodeling during hyphal growth, but without any apparent deletion phenotype. In this case mutants with multiple deletions might be necessary to reveal underlying functions. *chit-1* expression is upregulated in media containing colloidal chitin as the major carbon source, pointing towards a function in nutritional chitin acquisition in addition to its role in hyphal growth. Four chitinase genes are specifically induced during competitive interaction with *Fusarium sporotrichoides*, although the biological relevance of this data remains

elusive. In mycoparasitic fungi such as *Clonostachys rosea* and *T. atroviride* it was shown that the chitinases *ech42* and *ech37* are induced in the presence of exogenous chitin, *N*-acetylglucosamine (NAG), and fungal cell walls (Mach et al. 1999; Seidl et al. 2005; de las Mercedes Dana et al. 2001), and a direct role in mycoparasitism was suggested for *Ta*Ech42 (Mamarabadi et al. 2008; Carsolio et al. 1999). Interestingly the regulation of *ech42* in *Trichoderma* also depends on the GH20-gene *nag1* (Brunner et al. 2003) indicating a feedback-loop in which the degradation of chitin leads to the production of NAG, which might be taken up and trigger further upregulation of chitinolytic enzymes. In addition, expression of these chitinases is regulated through carbon catabolite-repression in the presence of glucose (Lorito et al. 1996; Mamarabadi et al. 2009).

Another mechanism for spatio-temporal regulation of chitinases has been described in *S. cerevisiae*. As mentioned above *Sc*Cts1p is essential for cell separation after cytokinesis (Kuranda and Robbins 1991), by degrading chitin of the primary septum. Therefore *Cts1* expression gets activated specifically in daughter cells during late M-phase and the protein subsequently dynamically localizes to the daughter site of the septum (Fig. 2a; Colman-Lerner et al. 2001). This tight regulation requires the concerted action of the FEAR (CDC14 early anaphase release), mitotic exit network (MEN), and regulation of Ace2p and morphogenesis (RAM) networks. During mitosis activation of FEAR and MEN leads to the release of the phosphatase Cdc14 from the nucleolus/nucleus into the cytoplasm where it counteracts cyclin-dependent kinase (Cdk1) activity to initiate cytokinesis (reviewed in Stegmeier and Amon 2004; Wurzenberger and Gerlich 2011). Furthermore, Cdc14 dephosphorylates the Cbk1p–Mob2p complex and the transcription factor Ace2p to promote their translocation into the daughter cell nucleus (for details see Sanchez-Diaz et al. 2012; Brace et al. 2010; Mancini Lombardi et al. 2013). Here the Cbk1p-Mob2p complex activates Ace2p to initiate the transcription of downstream genes, including the chitinase gene *CTS1* and the glucanase gene *ENG1* (Baladrón et al. 2002; Colman-Lerner et al. 2001; Nelson 2003). Thus, the integration of different regulatory networks enables cell cycle dependent, daughter cell-specific regulation of cell wall degrading enzymes.

In *U. maydis* two chitinases, *Um*Cts1 and *Um*Cts2, act redundantly in cell separation. Remarkably, the third GH18 chitinase, *Um*Cts3, is not expressed and hence does not contribute to chitinolytic activity during yeast-like or filamentous growth suggesting a specialized function, e.g., in competition with other fungi or nutrient acquisition, which yet remains to be identified (Langner et al. 2015). The major chitinolytic activity in saprotrophic cultures is attributed to *Um*Cts1, which is expressed during both yeast-like and filamentous growth. During cell division *Um*Cts1 dynamically localizes to the division zone in a cell cycle dependent, daughter cell-specific manner (Fig. 2b, c; Langner et al. 2015). However, the exact mechanism which regulates *Um*Cts1 relocalization remains elusive. Furthermore, it is yet unclear if *cts1* expression is differentially regulated in daughter cells and if a regulatory network reminiscent of the RAM network exists. During filamentous growth which represents the initial phase of pathogenic development of *U. maydis*, extracellular *Um*Cts1 activity is increased (Koepke et al. 2011). This increased activity is controlled by a transcriptional upregulation of *cts1* expression eight to twelve hours post filament induction (Heimel et al. 2010). Intriguingly, *Um*Cts2 does not contribute to the chitinolytic potential of *U. maydis* during filamentous growth. The loss of *Um*Cts2 activity in filaments is accompanied by a transcriptional downregulation in artificially induced filaments in axenic culture or on hydrophobic surfaces resembling the situation on the host leaf (Langner et al. 2015; Lanver et al. 2014). To date the functional relevance of this downregulation of *cts2* expression for polarized growth or infection of the host remains elusive. It is conceivable that *Um*Cts2 secretion into the apoplastic space during plant-pathogen interaction might be perceived by the host, either directly or indirectly through the production of CHOS, and could therefore elicit defense responses leading to immunity.

Secretion of chitinolytic enzymes

To act on the cell wall, chitinolytic enzymes essentially have to be secreted. In eukaryotes, most of them therefore contain a signal peptide which targets the respective protein to the endoplasmic reticulum (ER) for conventional secretion (Seidl et al. 2005; Langner et al. 2015). During the passage through the ER and the Golgi apparatus they often undergo post-translational modifications, like glycosylation or proteolytic cleavage (Tanaka et al. 2005; Gentzsch and Tanner 1997; Baladrón et al. 2002; Lange et al. 1996). Such processed proteins are then packed into secretory vesicles and transported to the plasma membrane for secretion. Interestingly however, some cell wall-associated proteins do not contain classical secretion peptides (Teparić et al. 2007; Chaffin 2008; Koepke et al. 2011) indicative of an unconventional, yet unknown secretion pathway (Steringer et al. 2015; Shoji et al. 2014). For example, the leaderless chitinase Cts1 of *U. maydis* avoids the classic route for secretion (Stock et al. 2012; Sarkari et al. 2014). Similarly, *Sc*Cts2p of *S. cerevisiae* and its orthologue *Ca*Cht4p of *C. albicans* do not contain classical secretion signals, but are still present in the cell wall (Teparić et al. 2007; Chaffin 2008). ER avoidance could occur for two reasons: One possibility is that post-translational modifications might render the enzyme inactive. Predicted *N*-glycosylation sites in the

leaderless chitinases *Sc*Cts2p, *Ca*Cht4p, and *Um*Cts1 support this idea. Secondly, chitinases might interfere with the glycosylation machinery by binding to GlcNAc-containing sugars in the ER. Such endo-ß-*N*-acetylglucosaminidase- or peptide-*N*-glycosidase-activities were shown for bacterial enzymes (Murata et al. 2005; Larsen et al. 2010; Frederiksen et al. 2013). *Um*Cts1 can also bind and process small CHOS (Langner et al. 2015) and might therefore interfere with the core sugar residues of *N*-glycosylated proteins in the ER similar to the bacterial enzymes. Thus, it is conceivable that a conserved alternative secretion pathway exists for secretion of chitinolytic enzymes to prevent the cell from severe damage due to false glycosylation and misfolding of proteins.

The role of chitin and chitinases in plant-fungus interactions

Phytopathogenic fungi take advantage of their host plant for completion of the life cycle. However, plants possess various defense mechanisms to counteract fungal infection. The first layer of the plant immune system involves recognition of microbe/pathogen-associated molecular pattern (MAMPs/PAMPs) to trigger basal defense responses (reviewed in Zipfel 2014; Sánchez-Vallet et al. 2014; Ben Khaled et al. 2015). Perception of MAMPs is mediated by highly conserved, extracellular transmembrane receptor proteins, called pattern-recognition receptors (PRRs) and receptor-like proteins (RLPs), which activate defense responses leading to MAMP-triggered immunity (Fig. 3; reviewed in Shinya et al. 2015; Tanaka et al. 2013). However, pathogens have evolved several mechanisms to evade the recognition and subsequent defense responses of the host in an evolutionary process often referred to as "molecular arms race" (Fig. 3; reviewed in Oliveira-Garcia and Valent 2015; Stergiopoulos and de Wit 2009).

Chitin or soluble CHOS are potent MAMPs that reveal the presence of fungal structures to the plant (Liu et al. 2012, 2014; Kombrink et al. 2011). CHOS binding by plant receptors is mediated through extracellular lysine motifs (LysM) containing PRRs and RLPs. The first immune receptor described in chitin reception was the chitin elicitor-binding protein (*Os*CEBIP) in rice (Kaku et al. 2006). Although CEBIP lacks a cytoplasmic kinase domain, it is directly involved in chitin-triggered immunity (Hayafune et al. 2014; Kouzai et al. 2013). *Os*CEBIP requires long CHOS, such as chito-octamers, to activate immune responses, most probably through homodimerization. Subsequently, the *Os*CEBIP homodimer recruits the chitin elicitor receptor kinase 1 (*Os*CERK1) which triggers phosphorylation of its intracellular kinase domain (Hayafune et al. 2014; Liu et al. 2012). Activated

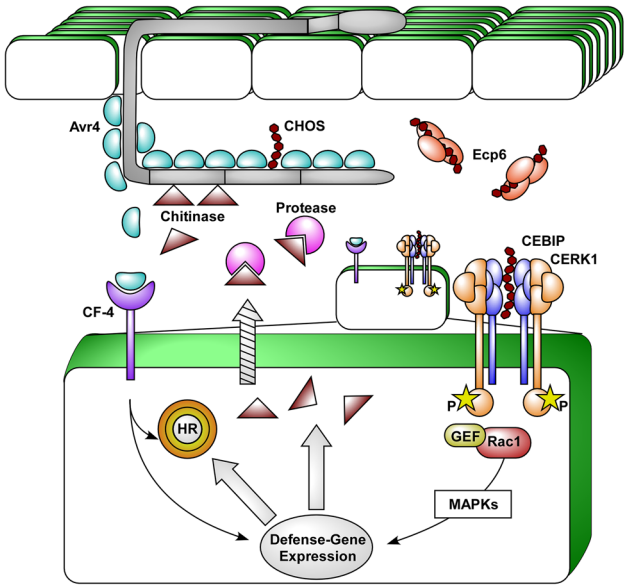

Fig. 3 Chitin triggered immunity in plants and evasion strategies of fungal pathogens. After entry into the host plant, the fungal cell wall is attacked by host secreted chitinases, which liberate soluble chito-oligosaccharides (CHOS) into the apoplastic space. CHOS with a chain length of $n \geq 8$ are bound by the plant LysM containing receptor-like protein (RLP) CEBIP. Subsequently, the chitin elicited receptor kinase 1 (CERK1) gets recruited which in turn leads to phosphorylation of its intracellular kinase domain. Activated CERK1 subsequently phosphorylates the Rac1 specific guanine exchange factor 1 (Rac1GEF1), which in turn activates a MAPK cascade that triggers expression of defense-genes, including hydrolytic enzymes. To protect the cell wall and to avoid recognition by the host, fungal pathogens secrete effector proteins to the apoplast. One such effector is Avr4 which binds to chitin and protects the cell wall from host-derived chitinases. Avr4 in turn can be perceived by the plant receptor CF-4 inducing defense-gene expression and hypersensitive response (HR). Therefore, fungi employ alternative strategies to evade host immunity. Fungus-derived proteases are secreted into the apoplastic space to degrade host chitinases and thereby counteracting the hydrolytic attack. To avoid CHOS recognition fungi can also secrete CHOS-binding effectors, like Ecp6. Ecp6 binds to CHOS with ultra-high affinity, thereby outcompeting binding by plant receptors and preventing recognition

*Os*CERK1 phosphorylates the Rac1 guanine nucleotide exchange factor 1 (*Os*RacGEF1) which leads to the activation of MAPK cascades through the small GTPase Rac1 (Fig. 3; Akamatsu et al. 2013; Shimizu et al. 2010). A similar pathway was also proposed in *Arabidopsis thaliana* which involves interaction of *At*CERK1 with the LysM RLK *At*LYK5 (Cao et al. 2014). As a result, defense responses are triggered which include the production of reactive oxygen species (ROS) and the expression of defense-related proteins, such as hydrolytic enzymes (Miya et al. 2007; Su et al. 2015).

To protect their cell wall from host chitinases or to prevent CHOS-mediated receptor activation, fungi have evolved multiple strategies. In the leaf mold *Cladosporium*

fulvum expression of the apoplastic effector *Cf*Avr4 enhances virulence (van Esse et al. 2007). *Cf*Avr4 is a chitin-binding lectin that is secreted during infection and protects the integrity of the fungal cell wall against plant chitinases (van den Burg et al. 2006). However, this protective mechanism is only functional in host plants which do not carry CF-4, a membrane-anchored LRR-RLP that recognizes Avr4 and subsequently triggers a hypersensitive response (HR; Fig. 3; Stergiopoulos et al. 2010). However, many successful pathogens, e.g., *F. oxysporum* or *Verticillium dahliae* do not encode homologs of *avr4* in their genomes. Nevertheless, most of them are still able to suppress degradation of their cell wall by plant chitinases, suggesting alternative strategies to counteract chitinase activity.

In line with this, *F. oxysporum* as well as the maize pathogens *F. verticillioides* and *Bipolaris zeicola* employ such a second strategy which involves the secretion of proteases acting against antifungal plant chitinases (Jashni et al. 2015; Naumann and Price 2012; Naumann et al. 2009). These proteins, including metallo- and serine-proteases, specifically cleave plant chitinases, reducing their antifungal activity, and thus, promote virulence of the pathogen (Fig. 3; Jashni et al. 2015).

Alternatively, fungi can evade recognition by the host through the secretion of apoplastic LysM effectors, like *Mo*Slp1 of the rice blast fungus *Magnaporthe oryza* or *Cf*Ecp6 of *C. fulvum* (Mentlak et al. 2012; de Jonge et al. 2010). *Cf*Ecp6 can bind to soluble CHOS with ultra-high affinity through intrachain dimerization of two LysM domains (Sánchez-Vallet et al. 2013). Thereby *Cf*Ecp6 is able to out-compete binding of CHOS to plant PRRs and hence suppresses chitin-triggered immunity (Fig. 3).

The above mentioned strategies to mediate and avoid chitin recognition exemplify the extent of co-evolution of virulence and defense mechanisms in plant/fungus interactions. Although huge progress has been made in the last decades, our knowledge of immunity induced by apoplastic molecules is still fragmented. How did fungal pathogens adapt to the perception and induction of immune responses by the host? Can pathogen-derived chitinases, which remodel the cell wall, also act as elicitors either through direct interaction with plant receptors/R-proteins or indirectly by releasing CHOS to the apoplast? How do phytopathogenic fungi employ their chitinases in competition with other fungi of the microbiome while protecting their own cell wall?

Concluding remarks and outlook

Investigation of plant infectious fungal model species has shed light on virulence and defense mechanisms related to chitin. However, little is known about the role of fungal chitinases during interaction with the host plant. Recently we showed that two chitinases, *Um*Cts1 and *Um*Cts2, are involved in cell wall remodeling in the corn smut *U. maydis* (Langner et al. 2015). Both enzymes differ in their binding/cleavage-site specificity, suggesting that *Um*Cts1 might have a higher affinity against short CHOS, whereas *Um*Cts2 might release longer soluble fragments. Interestingly, *cts2* transcription is repressed during early infection stages, but the biological importance of this downregulation is still unknown. It is conceivable that *Um*Cts2 activity needs to be repressed to prevent release of CHOS acting as MAMPs *in planta*. Alternatively, apoplastic *Um*Cts2 itself might be recognized by plant receptors, and thus, trigger immunity. Conversely, it can be speculated that fungal apoplastic chitinolytic enzymes might degrade CHOS as a counter-defense strategy, although experimental evidence for this is still lacking. In this context it would be interesting to investigate the precise role of fungal chitinases during intimate contact of the pathogen and its host plant.

There is still a shortage in model pathosystems to investigate chitinase function and chitin perception by the host at the same time during biotrophic interactions. A promising model system might be the biotrophic smut fungus *Thecaphora thlaspeos* which infects *Arabis* spp. in nature. Future progress in this field will show which roles chitin and/or chitinase perception play during biotrophic interactions and to which extend co-adaptation of the pathogen and the host plant enables invasion and colonization of the host. Such insights will support our understanding of beneficial versus detrimental interactions and could support resistance breeding in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adams DJ (2004) Fungal cell wall chitinases and glucanases. Microbiology 150:2029–2035

Akamatsu A, Wong Hann L, Fujiwara M, Okuda J, Nishide K, Uno K, Imai K, Umemura K, Kawasaki T, Kawano Y et al (2013) An OsCEBiP/OsCERK1-OsRacGEF1-OsRac1 module is an essential early component of chitin-induced rice immunity. Cell Host Microbe 13:465–476

Baker LG, Specht CA, Lodge JK (2009) Chitinases are essential for sexual development but not vegetative growth in *Cryptococcus neoformans*. Eukaryot Cell 8:1692–1705

Baladrón V, Ufano S, Duenas E, Martin-Cuadrado AB, del Rey F, Vazquez de Aldana CR (2002) Eng1p, an endo-1,3-β-glucanase

localized at the daughter side of the septum, is involved in cell separation in *Saccharomyces cerevisiae*. Eukaryot Cell 1:774–786

Beeson WT, Vu VV, Span EA, Phillips CM, Marletta MA (2015) Cellulose degradation by polysaccharide monooxygenases. Annu Rev Biochem 84:923–946

Ben Khaled S, Postma J, Robatzek S (2015) A moving view: subcellular trafficking processes in pattern recognition receptor-triggered plant immunity. Annu Rev Phytopathol 53:379–402

Bowen AR, Chen-Wu JL, Momany M, Young R, Szaniszlo PJ, Robbins PW (1992) Classification of fungal chitin synthases. Proc Natl Acad Sci USA 89:519–523

Bowman SM, Free SJ (2006) The structure and synthesis of the fungal cell wall. BioEssays 28:799–808

Brace J, Hsu J, Weiss EL (2010) Mitotic exit control of the *Saccharomyces cerevisiae* Ndr/LATS kinase Cbk1 regulates daughter cell separation after cytokinesis. Mol Cell Biol 31:721–735

Brunner K, Peterbauer CK, Mach RL, Lorito M, Zeilinger S, Kubicek CP (2003) The Nag1 *N*-acetylglucosaminidase of Trichoderma atroviride is essential for chitinase induction by chitin and of major relevance to biocontrol. Curr Genet 43:289–295

Cabib E, Arroyo J (2013) How carbohydrates sculpt cells: chemical control of morphogenesis in the yeast cell wall. Nat Rev Microbiol 11:648–655

Cabib E, Blanco N, Grau C, Rodriguez-Pena JM, Arroyo J (2007) Crh1p and Crh2p are required for the cross-linking of chitin to beta(1-6)glucan in the *Saccharomyces cerevisiae* cell wall. Mol Microbiol 63:921–935

Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, Joachimiak A, Stacey G (2014) The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. Elife 3:e03766

Carsolio C, Gutierrez A, Jimenez B, Van Montagu M, Herrera-Estrella A (1994) Characterization of ech-42, a *Trichoderma harzianum* endochitinase gene expressed during mycoparasitism. Proc Natl Acad Sci USA 91:10903–10907

Carsolio C, Benhamou N, Haran S, Cortes C, Gutierrez A, Chet I, Herrera-Estrella A (1999) Role of the *Trichoderma harzianum* endochitinase gene, *ech42*, in mycoparasitism. Appl Environ Microbiol 65:929–935

Chaffin WL (2008) *Candida albicans* cell wall proteins. Microbiol Mol Biol Rev 72:495–544

Chen F, Chen X-Z, Qin L-N, Tao Y, Dong Z-Y (2015) Characterization and homologous overexpression of an *N*-acetylglucosaminidase Nag1 from *Trichoderma reesei*. Biochem Biophys Res Commun 459:184–188

Colman-Lerner A, Chin TE, Brent R (2001) Yeast Cbk1 and Mob2 activate daughter-specific genetic programs to induce asymmetric cell fates. Cell 107:739–750

Colussi PA, Specht CA, Taron CH (2005) Characterization of a nucleus-encoded chitinase from the yeast *Kluyveromyces lactis*. Appl Environ Microbiol 71:2862–2869

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

de Jonge R, van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, van der Krol S, Shibuya N, Joosten MH, Thomma BP (2010) Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science 329:953–955

de las Mercedes Dana M, Limón MC, Mejías R, Mach RL, Benítez T et al (2001) Regulation of chitinase 33 (chit33) gene expression in *Trichoderma harzianum*. Curr Genet 38:335–342

Drouillard S, Armand S, Davies GJ, Vorgias CE, Henrissat B (1997) *Serratia marcescens* chitobiase is a retaining glycosidase utilizing substrate acetamido group participation. Biochem J 328(Pt 3):945–949

Dünkler A, Walther A, Specht CA, Wendland J (2005) *Candida albicans* CHT3 encodes the functional homolog of the Cts1 chitinase of *Saccharomyces cerevisiae*. Fungal Genet Biol 42:935–947

Dünkler A, Jorde S, Wendland J (2008) An *Ashbya gossypii* cts2 mutant deficient in a sporulation-specific chitinase can be complemented by *Candida albicans* CHT4. Microbiol Res 163:701–710

Durán A, Bowers B, Cabib E (1975) Chitin synthetase zymogen is attached to the yeast plasma membrane. Proc Natl Acad Sci USA 72:3952–3955

Egan MJ, McClintock MA, Reck-Peterson SL (2012) Microtubule-based transport in filamentous fungi. Curr Opin Microbiol 15:637–645

Eijsink VGH, Vaaje-Kolstad G, Vårum KM, Horn SJ (2008) Towards new enzymes for biofuels: lessons from chitinase research. Trends Biotechnol 26:228–235

Feldbrügge M, Kämper J, Steinberg G, Kahmann R (2004) Regulation of mating and pathogenic development in *Ustilago maydis*. Curr Opin Microbiol 7:666–672

Frederiksen RF, Paspaliari DK, Larsen T, Storgaard BG, Larsen MH, Ingmer H, Palcic MM, Leisner JJ (2013) Bacterial chitinases and chitin-binding proteins as virulence factors. Microbiology 159:833–847

García I, Jimenez D, Martin V, Duran A, Sanchez Y (2005) The alpha-glucanase Agn1p is required for cell separation in *Schizosaccharomyces pombe*. Biol Cell 97:569–576

Gentzsch M, Tanner W (1997) Protein-*O*-glycosylation in yeast: protein-specific mannosyltransferases. Glycobiology 7:481–486

Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B et al (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 418:387–391

Gruber S, Seidl-Seiboth V (2011) Self versus non-self: fungal cell wall degradation in Trichoderma. Microbiology 158:26–34

Gruber S, Kubicek CP, Seidl-Seiboth V (2011) Differential regulation of Orthologous chitinase genes in mycoparasitic Trichoderma species. Appl Environ Microbiol 77:7217–7226

Hartl L, Zach S, Seidl-Seiboth V (2012) Fungal chitinases: diversity, mechanistic properties and biotechnological potential. Appl Microbiol Biotechnol 93:533–543

Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, Desaki Y, Arima S, Squeglia F, Ruggiero A, Tokuyasu K et al (2014) Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. Proc Natl Acad Sci USA 111:E404–E413

Heimel K, Scherer M, Schuler D, Kamper J (2010) The *Ustilago maydis* Clp1 protein orchestrates pheromone and b-dependent signaling pathways to coordinate the cell cycle and pathogenic development. Plant Cell 22:2908–2922

Henrissat B (1991) A classification of glycosyl hydrolases based on amino-acid-sequence similarities. Biochem J 280:309–316

Horn SJ, Sorbotten A, Synstad B, Sikorski P, Sorlie M, Varum KM, Eijsink VGH (2006) Endo/exo mechanism and processivity of family 18 chitinases produced by *Serratia marcescens*. FEBS J 273:491–503

Hours RA, Gortari MC (2013) Biotechnological processes for chitin recovery out of crustacean waste: a mini-review. Electron J Biotechnol 16:14

Ichinomiya M, Yamada E, Yamashita S, Ohta A, Horiuchi H (2005) Class I and class II chitin synthases are involved in septum formation in the filamentous fungus *Aspergillus nidulans*. Eukaryot Cell 4:1125–1136

Igarashi K, Uchihashi T, Koivula A, Wada M, Kimura S, Okamoto T, Penttila M, Ando T, Samejima M (2011) Traffic jams reduce hydrolytic efficiency of cellulase on cellulose surface. Science 333:1279–1282

Jashni MK, Dols IH, Iida Y, Boeren S, Beenen HG, Mehrabi R, Collemare J, de Wit PJ (2015) Synergistic action of a metalloprotease and a serine protease from *Fusarium oxysporum* f. sp. *lycopersici* cleaves chitin-binding tomato chitinases, reduces their antifungal activity, and enhances fungal virulence. Mol Plant Microbe Interact 28:996–1008

Jones CS, Kosman DJ (1980) Purification, properties, kinetics, and mechanism of beta-*N*-acetylglucosamidase from *Aspergillus niger*. J Biol Chem 255:11861–11869

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc Natl Acad Sci 103:11086–11091

Kämper J, Kahmann R, Bölker M, Ma L-J, Brefort T, Saville BJ, Banuett F, Kronstad JW, Gold SE, Müller O et al (2006) Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. Nature 444:97–101

Karlsson M, Stenlid J (2008) Comparative evolutionary histories of the fungal chitinase gene family reveal non-random size expansions and contractions due to adaptive natural selection. Evol Bioinform Online 4:47–60

Kim JE, Lee HJ, Lee J, Kim KW, Yun SH, Shim WB, Lee YW (2009) Gibberella zeae chitin synthase genes, GzCHS5 and GzCHS7, are required for hyphal growth, perithecia formation, and pathogenicity. Curr Genet 55:449–459

Koepke J, Kaffarnik F, Haag C, Zarnack K, Luscombe NM, Konig J, Ule J, Kellner R, Begerow D, Feldbrugge M (2011) The RNA-binding protein Rrm4 is essential for efficient secretion of endochitinase Cts1. Mol Cell Proteomics 10:M111-011213

Kombrink A, Sanchez-Vallet A, Thomma BP (2011) The role of chitin detection in plant—pathogen interactions. Microbes Infect 13:1168–1176

Kouzai Y, Nakajima K, Hayafune M, Ozawa K, Kaku H, Shibuya N, Minami E, Nishizawa Y (2013) CEBiP is the major chitin oligomer-binding protein in rice and plays a main role in the perception of chitin oligomers. Plant Mol Biol 84:519–528

Kuranda MJ, Robbins PW (1991) Chitinase is required for cell separation during growth of *Saccharomyces cerevisiae*. J Biol Chem 266:19758–19767

Lange J, Mohr U, Wiemken A, Boller T, Vogeli-Lange R (1996) Proteolytic processing of class IV chitinase in the compatible interaction of bean roots with *Fusarium solani*. Plant Physiol 111:1135–1144

Langner T, Öztürk M, Hartmann S, Cord-Landwehr S, Moerschbacher B, Walton JD, Göhre V (2015) Chitinases are essential for cell separation in *Ustilago maydis*. Eukaryot Cell 14:846–857

Lanver D, Berndt P, Tollot M, Naik V, Vranes M, Warmann T, Münch K, Rössel N, Kahmann R (2014) Plant surface cues prime *Ustilago maydis* for biotrophic development. PLoS Pathog 10:e1004272

Larsen T, Petersen BO, Storgaard BG, Duus JO, Palcic MM, Leisner JJ (2010) Characterization of a novel Salmonella Typhimurium chitinase which hydrolyzes chitin, chitooligosaccharides and an *N*-acetyllactosamine conjugate. Glycobiology 21:426–436

Latgé J-P (2007) The cell wall: a carbohydrate armour for the fungal cell. Mol Microbiol 66:279–290

Leake JR, Read DJ (1990) Chitin as a Nitrogen-source for mycorrhizal fungi. Mycol Res 94:993–995

Lenardon MD, Munro CA, Gow NA (2010) Chitin synthesis and fungal pathogenesis. Curr Opin Microbiol 13:416–423

Levin DE (2011) Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. Genetics 189:1145–1175

Liu T, Liu Z, Song C, Hu Y, Han Z, She J, Fan F, Wang J, Jin C, Chang J et al (2012) Chitin-induced dimerization activates a plant immune receptor. Science 336:1160–1164

Liu X, Grabherr HM, Willmann R, Kolb D, Brunner F, Bertsche U, Kuhner D, Franz-Wachtel M, Amin B, Felix G et al (2014) Host-induced bacterial cell wall decomposition mediates pattern-triggered immunity in Arabidopsis. Elife 3:e01990

Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495

Lorito M, Mach RL, Sposato P, Strauss J, Peterbauer CK, Kubicek CP (1996) Mycoparasitic interaction relieves binding of the Cre1 carbon catabolite repressor protein to promoter sequences of the ech42 (endochitinase-encoding) gene in *Trichoderma harzianum*. Proc Natl Acad Sci USA 93:14868–14872

Mach RL, Peterbauer CK, Payer K, Jaksits S, Woo SL, Zeilinger S, Kullnig CM, Lorito M, Kubicek CP (1999) Expression of two major chitinase genes of Trichoderma atroviride (*T. harzianum* P1) is triggered by different regulatory signals. Appl Environ Microbiol 65:1858–1863

Maddi A, Bowman SM, Free SJ (2009) Trifluoromethanesulfonic acid-based proteomic analysis of cell wall and secreted proteins of the ascomycetous fungi *Neurospora crassa* and *Candida albicans*. Fungal Genet Biol 46:768–781

Mamarabadi M, Jensen B, Lübeck M (2008) Three endochitinase-encoding genes identified in the biocontrol fungus *Clonostachys rosea* are differentially expressed. Curr Genet 54:57–70

Mamarabadi M, Jensen DF, Lübeck M (2009) An *N*-acetyl-β-*d*-glucosaminidase gene, cr-nag1, from the biocontrol agent *Clonostachys rosea* is up-regulated in antagonistic interactions with *Fusarium culmorum*. Mycol Res 113:33–43

Mancini Lombardi I, Palani S, Meitinger F, Darieva Z, Hofmann A, Sharrocks Andrew D, Pereira G (2013) Lre1 directly inhibits the NDR/lats kinase Cbk1 at the cell division site in a phosphorylation-dependent manner. Curr Biol 23:1736–1745

Martin-Cuadrado AB (2003) The endo-beta-1,3-glucanase eng1p is required for dissolution of the primary septum during cell separation in *Schizosaccharomyces pombe*. J Cell Sci 116:1689–1698

Mathé L, Van Dijck P (2013) Recent insights into *Candida albicans* biofilm resistance mechanisms. Curr Genet 59:251–264

Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, Terauchi R, Nishizawa Y, Shibuya N, Thomma BPHJ et al (2012) Effector-mediated suppression of chitin-triggered immunity by *Magnaporthe oryzae* is necessary for rice blast disease. Plant Cell 24:322–335

Merzendorfer H (2003) Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. J Exp Biol 206:4393–4412

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc Natl Acad Sci 104:19613–19618

Murata T, Amarume S, Hattori T, Tokuyama S, Tokuyasu K, Kawagishi H, Usui T (2005) Purification and characterization of a chitinase from Amycolatopsis orientalis with *N*-acetyllactosamine-repeating unit releasing activity. Biochem Biophys Res Commun 336:514–520

Nakagawa YS, Kudo M, Loose JSM, Ishikawa T, Totani K, Eijsink VGH, Vaaje-Kolstad G (2015) A small lytic polysaccharide monooxygenase from *Streptomyces griseus* targeting α- and β-chitin. FEBS J 282:1065–1079

Naumann TA, Price NPJ (2012) Truncation of class IV chitinases from Arabidopsis by secreted fungal proteases. Mol Plant Pathol 13:1135–1139

Naumann TA, Wicklow DT, Kendra DF (2009) Maize seed chitinase is modified by a protein secreted by *Bipolaris zeicola*. Physiol Mol Plant Pathol 74:134–141

Nelson B (2003) RAM: a conserved signaling network that regulates Ace2p transcriptional activity and polarized morphogenesis. Mol Biol Cell 14:3782–3803

Oliveira-Garcia E, Valent B (2015) How eukaryotic filamentous pathogens evade plant recognition. Curr Opin Microbiol 26:92–101

Patil RS, Ghormade VV, Deshpande MV (2000) Chitinolytic enzymes: an exploration. Enzyme Microb Technol 26:473–483

Phillips CM, Beeson WT, Cate JH, Marletta MA (2011) Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by *Neurospora crassa*. ACS Chem Biol 6:1399–1406

Roncero C (2002) The genetic complexity of chitin synthesis in fungi. Curr Genet 41:367–378

Ruiz-Herrera J, Gonzalez-Prieto JM, Ruiz-Medrano R (2002) Evolution and phylogenetic relationships of chitin synthases from yeasts and fungi. FEMS Yeast Res 1:247–256

Sanchez-Diaz A, Nkosi PJ, Murray S, Labib K (2012) The mitotic exit network and Cdc14 phosphatase initiate cytokinesis by counteracting CDK phosphorylations and blocking polarised growth. EMBO J 31:3620–3634

Sánchez-Vallet A, Saleem-Batcha R, Kombrink A, Hansen G, Valkenburg DJ, Thomma BP, Mesters JR (2013) Fungal effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. Elife 2:e00790

Sánchez-Vallet A, Mesters JR, Thomma BPHJ (2014) The battle for chitin recognition in plant-microbe interactions. FEMS Microbiol Rev 39:171–183

Sarkari P, Reindl M, Stock J, Muller O, Kahmann R, Feldbrugge M, Schipper K (2014) Improved expression of single-chain antibodies in *Ustilago maydis*. J Biotechnol 191:165–175

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

Seidl V, Huemer B, Seiboth B, Kubicek CP (2005) A complete survey of Trichoderma chitinases reveals three distinct subgroups of family 18 chitinases. FEBS J 272:5923–5939

Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, Minami E, Okada K, Yamane H, Kaku H et al (2010) Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. Plant J 64:204–214

Shin KS, Kwon NJ, Kim YH, Park HS, Kwon GS, Yu JH (2009) Differential roles of the ChiB chitinase in autolysis and cell death of *Aspergillus nidulans*. Eukaryot Cell 8:738–746

Shinya T, Nakagawa T, Kaku H, Shibuya N (2015) Chitin-mediated plant–fungal interactions: catching, hiding and handshaking. Curr Opin Plant Biol 26:64–71

Shoji J-Y, Kikuma T, Kitamoto K (2014) Vesicle trafficking, organelle functions, and unconventional secretion in fungal physiology and pathogenicity. Curr Opin Microbiol 20:1–9

Sietsma JH, Beth Din A, Ziv V, Sjollema KA, Yarden O (1996) The localization of chitin synthase in membranous vesicles (chitosomes) in *Neurospora crassa*. Microbiology 142(Pt 7):1591–1596

Silverman SJ, Sburlati A, Slater ML, Cabib E (1988) Chitin synthase 2 is essential for septum formation and cell division in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 85:4735–4739

Starr TL, Pagant S, Wang CW, Schekman R (2012) Sorting signals that mediate traffic of chitin synthase III between the TGN/endosomes and to the plasma membrane in yeast. PLoS One 7:e46386

Stegmeier F, Amon A (2004) Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. Annu Rev Genet 38:203–232

Stergiopoulos I, de Wit PJGM (2009) Fungal effector proteins. Annu Rev Phytopathol 47:233–263

Stergiopoulos I, van den Burg HA, Okmen B, Beenen HG, van Liere S, Kema GHJ, de Wit PJGM (2010) Tomato Cf resistance proteins mediate recognition of cognate homologous effectors from fungi pathogenic on dicots and monocots. Proc Natl Acad Sci 107:7610–7615

Steringer JP, Muller HM, Nickel W (2015) Unconventional secretion of fibroblast growth factor 2—a novel type of protein translocation across membranes? J Mol Biol 427:1202–1210

Stock J, Sarkari P, Kreibich S, Brefort T, Feldbrugge M, Schipper K (2012) Applying unconventional secretion of the endochitinase Cts1 to export heterologous proteins in *Ustilago maydis*. J Biotechnol 161:80–91

Su Y, Xu L, Wang S, Wang Z, Yang Y, Chen Y, Que Y (2015) Identification, phylogeny, and transcript of chitinase family genes in sugarcane. Sci Rep 5:10708

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

Tanaka N, Fujita Y, Suzuki S, Morishita M, Giga-Hama Y, Shimoda C, Takegawa K (2005) Characterization of *O*-mannosyltransferase family in *Schizosaccharomyces pombe*. Biochem Biophys Res Commun 330:813–820

Tanaka K, Nguyen CT, Liang Y, Cao Y, Stacey G (2013) Role of LysM receptors in chitin-triggered plant innate immunity. Plant Signal Behav 8:e22598

Teparić R, Mrša V (2013) Proteins involved in building, maintaining and remodeling of yeast cell walls. Curr Genet 59:171–185

Teparić R, Stuparević I, Mrša V (2007) Binding assay for incorporation of alkali-extractable proteins in the *Saccharomyces cerevisiae* cell wall. Yeast 24:259–266

Terwisscha van Scheltinga AC, Armand S, Kalk KH, Isogai A, Henrissat B, Dijkstra BW (1995) Stereochemistry of chitin hydrolysis by a plant chitinase/lysozyme and X-ray structure of a complex with allosamidin evidence for substrate assisted catalysis. Biochemistry 34:15619–15623

Tews I, Vincentelli R, Vorgias CE (1996) *N*-Acetylglucosaminidase (chitobiase) from *Serratia marcescens*: gene sequence, and protein production and purification in *Escherichia coli*. Gene 170:63–67

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

Tzelepis GD, Melin P, Jensen DF, Stenlid J, Karlsson M (2012) Functional analysis of glycoside hydrolase family 18 and 20 genes in *Neurospora crassa*. Fungal Genet Biol 49:717–730

Vaaje-Kolstad G, Houston DR, Riemen AHK, Eijsink VGH, van Aalten DMF (2005) Crystal structure and binding properties of the *Serratia marcescens* chitin-binding protein CBP21. J Biol Chem 280:11313–11319

Vaaje-Kolstad G, Westereng B, Horn SJ, Liu Z, Zhai H, Sorlie M, Eijsink VG (2010) An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. Science 330:219–222

Vaaje-Kolstad G, Bøhle LA, Gåseidnes S, Dalhus B, Bjørås M, Mathiesen G, Eijsink VGH (2012) Characterization of the chitinolytic machinery of *Enterococcus faecalis* V583 and high-resolution structure of its oxidative CBM33 enzyme. J Mol Biol 416:239–254

Vaaje-Kolstad G, Horn SJ, Sørlie M, Eijsink VGH (2013) The chitinolytic machinery of *Serratia marcescens*—a model system for enzymatic degradation of recalcitrant polysaccharides. FEBS J 280:3028–3049

Valdivia RH, 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

van Aalten DMF, Komander D, Synstad B, Gaseidnes S, Peter MG, Eijsink VGH (2001) Structural insights into the catalytic mechanism of a family 18 exo-chitinase. Proc Natl Acad Sci 98:8979–8984

van den Burg HA, Harrison SJ, Joosten MH, Vervoort J, de Wit PJ (2006) Cladosporium fulvum Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. Mol Plant Microbe Interact 19:1420–1430

van Esse HP, Bolton MD, Stergiopoulos I, de Wit PJ, Thomma BP (2007) The chitin-binding *Cladosporium fulvum* effector protein Avr4 is a virulence factor. Mol Plant Microbe Interact 20:1092–1101

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

Williams SJ, Mark BL, Vocadlo DJ, James MNG, Withers SG (2002) Aspartate 313 in the *Streptomyces plicatus* hexosaminidase plays a critical role in substrate-assisted catalysis by orienting the 2-acetamido group and stabilizing the transition state. J Biol Chem 277:40055–40065

Wurzenberger C, Gerlich DW (2011) Phosphatases: providing safe passage through mitotic exit. Nat Rev Mol Cell Biol 12:469–482

Yamazaki H, Yamazaki D, Takaya N, Takagi M, Ohta A, Horiuchi H (2007) A chitinase gene, *chiB*, involved in the autolytic process of *Aspergillus nidulans*. Curr Genet 51:89–98

Yamazaki H, Tanaka A, Kaneko J-I, Ohta A, Horiuchi H (2008) Aspergillus nidulans ChiA is a glycosylphosphatidylinositol (GPI)-anchored chitinase specifically localized at polarized growth sites. Fungal Genet Biol 45:963–972

Zakariassen H, Aam BB, Horn SJ, Varum KM, Sorlie M, Eijsink VGH (2009) Aromatic residues in the catalytic center of chitinase A from *Serratia marcescens* affect processivity, enzyme activity, and biomass converting efficiency. J Biol Chem 284:10610–10617

Zakariassen H, Hansen MC, Joranli M, Eijsink VG, Sorlie M (2011) Mutational effects on transglycosylating activity of family 18 chitinases and construction of a hypertransglycosylating mutant. Biochemistry 50:5693–5703

Zipfel C (2014) Plant pattern-recognition receptors. Trends Immunol 35:345–351