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Review

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 (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 37 °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.

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 1–2 % of the 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 (10–20 % 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).

Abbreviations: Chs, Chitin synthase; MMD-Chs, Myosin Motor Domain – Chitin synthases.

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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 et al., 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 1, 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 subdivisions of classes V and VII Chs have been proposed, based on the amino acid sequence. We will follow the classification suggested by Roncero (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).

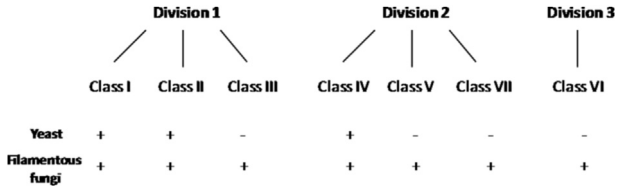

Fig. 1 – Categorization of Chs enzymes and distribution among yeast and filamentous fungi.

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 mollusks (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).