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REVIEW ARTICLE

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The genetic complexity of chitin synthesis in fungi

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Abstract Chitin synthesis is a process maintained across the fungal kingdom that, thanks to the power of genetic manipulation of yeast cells, is now beginning to be understood. Chitin synthesis is based on the regulation of distinct chitin synthase isoenzymes whose number ranges from one in *Schizosaccharomyces pombe* to seven in some filamentous fungi, such as *Aspergillus fumigatus*. This high diversity makes it difficult to find a unique model of regulation. However, the results available suggest common themes in regulation. The arrival of the genomic era, together with the development of fungal genetic technology should allow experimental approaches to this process.

Keywords Cell wall · Chitin · Fungi · Chitin synthases · CHS genes

Introduction

In nature, fungal cell life depends on an extracellular structure, the cell wall. This provides skeletal support to fungal cells, at the same time allowing the interaction of such cells with the surrounding environment. The integrity of this structure is essential for cell survival in hostile environments; and fungal cells devoid of cell wall – the so-called fungal protoplasts – can only survive under laboratory conditions where the osmotic support that prevents cell lysis can be provided externally (for a review, see Orlean 1997). It is therefore not surprising that the cell wall is universally distributed in all fungal taxonomic groups. This broad distribution includes fungi responsible for several human and animal pathologies, which became notorious in the past two decades because of the increased number of immunocompromised hosts, where these pathologies primarily develop. Accordingly, the fungal cell wall was used as a primary target for the development of new antifungal agents, some of which are likely to be marketed in the near future (Georgopapadakou 2001; Tkacz and DiDomenico 2001).

The fungal cell wall varies in composition and in structure between different groups; and several decades ago these differences were used for taxonomic classification (Bartnicki-Garcia 1968). Today, although this classification is no longer in use, it still reflects major differences between fungal groups that help to understand some of the hypotheses developed in this review article. Essentially, two types of molecules form fungal cell walls: fibrous polymers and gel-like polymers. The former constitute the structural skeleton of walls, while the gel-like molecules act as interconnecting molecules. The variations in the relationship between both types of molecules guarantee the dynamic properties of the fungal wall required for survival under different environmental conditions (for a recent review, see Smits et al. 2001).

This review does not attempt to address the fungal cell wall per se, a matter that was reviewed recently (Smits et al. 1999, 2001), but rather one of the fibrillar components of this structure: the chitin polymer. Chitin is a linear polymer of *N*-acetyl-glucosamine. It is crystalline and extraordinarily strong, with a tensile strength much greater than that of many artificial materials. This strength is the result of extensive hydrogen bonding along the chains while they are being formed. The importance of chitin in cell wall architecture is well documented and it was described several decades ago that inhibition of chitin synthesis produces cell death (for a review, see Cabib et al. 1996). This polymer appears to be widely distributed in the fungal kingdom, since nearly all fungi have significant amounts of chitin in their cell walls (Bartnicki-Garcia 1968).

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At the cellular level, chitin is the result of the activity of an enzyme called chitin synthase (CS). This was originally described in the late 1950s, but its corresponding gene was described in 1986 in the yeast *Saccharomyces cerevisiae* (Cabib et al. 1996). Since then, many more CS genes of fungal origin have been described. Recently, the presence of CS-like genes was reported in other evolutionary groups, such as insects, bacteria, protozoa and even vertebrates (Bulawa and Wasco 1991; Gagou et al. 2002; Semino et al. 1996).

This review does not aim at offering an evolutionary study of fungi, or an extensive review of chitin synthesis. Instead, based on the functional and genomic data collected so far, it attempts to explain the enormous diversity of CSs and the complex regulatory mechanisms involved in their control.

The CS genes in fungi

S. cerevisiae contains three CSs

From the 1950s to the mid-1980s, a considerable amount of information was collected about the biochemical properties of CSs from different fungi. Many such properties are strain-specific, but interestingly most of those synthases were reported as zymogenic enzymes, being localized in the plasma membrane. These results led to a regulation model based on the cellular compartmentalization of the inhibitors and activators of such activities (Cabib et al. 1982). However, this model was highly speculative, due to the intrinsic limitations of the biochemical approach. During the 1980s, Cabib's group began a search for the gene(s) that encodes yeast CS activity, the so-called *CHS* genes. This screening was based on the in vitro determination of CS from thousands of mutants and led to the identification of the *ScCHS1* gene, which encodes the catalytic subunit of the major in vitro CS activity. However, it very soon became apparent that this activity does not participate in the synthesis of cellular chitin (Bulawa et al. 1986). Further efforts led to the isolation of a second CS gene, through a screening based on the increased in vitro CS activity in the absence of *ScCHS1* (Silverman et al. 1988). This gene, *ScCHS2*, encodes a new CS activity (Sburlati and Cabib 1986), whose participation in chitin synthesis is minor, although its function is very important for cell survival (Bulawa and Osmond 1990). Both genes share a considerable amount of homology (Fig. 1a, b).

a

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ScCHS1  652 FEYKMSNILDKTTESNFGFITVLPGAFSAYRFEAVR-----GQPLQK--YFYGEIMENEG
ScCHS2  492 FEYKISNILDKPLESVFGYISVLPGALSAYRYRALKNHEDGTGPLRS--YFLGETQEGRD
ScCHS3  881 FEYYISHHQAKAFESVFGSVTCLPGCFSMYRIKSPKGSDGYWVPVLANPDIVERYSDNVT
            *** :*:   *. ** ** :: ***.:* ** .: :       *:     :  .  :.

ScCHS1      FHFFSSNMYLAEDRILCFEVVTKKNCNWILKYCRSSYASTDVPERVPEFILQRRRWLNG- 763
ScCHS2      HDVFTANMYLAEDRILCWELVAKRDAKWVLKYVKEATGETDVPEDVSEFISQRRRWLNG  608
ScCHS3      NTLHKKNLLLLGEDRFLSSLMLKTFPKRKQVFVPKAACKTIAPDKFKVLLSQRRRWINST 1000
              ... *: *  :  :  .:: *   :    :  .:  .* .*: .  :: *****:*.
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b

	1	2	3	4	5	6	7
1-*ScCHS1*	♦♦	29.6	28.9	37.9	32.4	9.7	10.8
2-*ScCHS2*		♦♦	42.3	32.0	30.3	11.6	10.1
3-*CaCHS1*			♦♦	32.7	29.7	10.7	10.9
4-*CaCHS2*				♦♦	38.3	10.8	10.4
5-*CaCHS8*					♦♦	10.5	10.1
6-*ScCHS3*						♦♦	56.7
7-*CaCHS3*							♦♦

Fig. 1a, b. Homologies among the different chitin synthases in yeast. **a** Clustal W alignment of the indicated fragments from the three *Saccharomyces cerevisiae* chitin synthase (CS) proteins. *Numbering* indicates the relative amino acid position inside each protein. *Asterisks* mark conserved amino acids and *single*/*double dots* mark conservative substitutions. *Underlined regions* show amino acid conservation between ScChs1p and ScChs2p (59% identity), which is significantly higher than that observed between the three proteins (22% identity, amino acids *in bold*). **b** Sequence identity between the complete CS proteins from *S. cerevisiae* and *Candida albicans*. *Each value* indicates the percentage of identity between two numbered proteins

In the late 1980s, two different approaches were used for the direct selection of mutants deficient in chitin synthesis in vivo. Our group selected for mutants resistant to calcofluor white, a drug that interacts directly with chitin, producing cell death (Roncero et al. 1988). At the same time, C.E. Bulawa selected for mutants deficient in chitin synthesis in vivo (Bulawa 1992). These screenings partly overlapped, leading to the isolation of several mutants deficient in chitin synthesis, from which the corresponding genes were isolated: *CHS3*, *CHS4*, *CHS5*, *CHS6* and *CHS7*. Most of them proved to be regulatory proteins, although the original *CHS* name was maintained. However, *CHS3* appears to encode a new and different CS protein (Valdivieso et al. 1991). This protein shows only limited homology with the other *S. cerevisiae* CS genes previously described, but contains a region of 120 amino acids with more than 22% identity (Fig. 1a). This region contains the QRRRW motif, which can be considered the signature motif for CSs (for a review, see Valdivieso et al. 1999), since it is essential for the catalytic activity (Cos et al. 1998). Further work with the null mutants of these genes allowed the identification in yeast of three different CS activities (Choi and Cabib 1994; Choi et al. 1994b), each with specific cellular functions. No further CS genes were found, after the complete sequencing of the *S. cerevisiae* genome.

A similar picture arose from the study of *Candida albicans*, where three genes (*CaCHS1*, *CaCHS2*, *CaCHS3*) were also promptly described. Recently, through the genomic approach, a fourth gene (*CaCHS8*) was described in this organism (for a review see Munro and Gow 2001).

Direct comparison of the *S. cerevisiae* and *C. albicans* *CHS* sequences points to the existence of two main groups of *CHS* genes (Fig. 1b). One of these groups includes the original *ScCHS1* gene, together with its homologous genes, with which it shares at least 30% identity. The other group includes *ScCHS3*, together with its *C. albicans* homologue, both highly conserved (56% identity) but significantly different from the other group.

Fungal genomes contain more than three *CHS* genes

Much more information about fungal *CHS* genes has emerged in recent years, due to the efforts of several groups attempting to identify the different *CHS* genes present in filamentous fungi. Most of these efforts were based on the identification of the conserved domain found in all CSs described (Fig. 1a). This high degree of conservation allowed the design of specific primers for PCR amplification. Today, more than 150 fungal *CHS* genes have been totally or partially sequenced, although to date only a few of them have been functionally characterized. Independently of their experimentally tested function, *CHS* gene sequences can be compared and aligned using different algorithms. Figure 2 shows a neighbour-joining tree of *CHS* proteins after Clustal W alignment (Thompson et al. 1994). This alignment is based on only some fungal genomes because a complete tree with more than 150 protein sequences would be very complicated. The sequences selected are from organisms about which we have more information concerning *CHS* functionality; and references will be cited later. However, the results obtained using both a partial set of sequences and the whole set are essentially the same (data not shown). Interestingly, *CHS*-like genes have also been found in insects (Gagou et al. 2002), nematodes (Veronico et al. 2001) and even bacteria (Bulawa and Wasco 1991). Therefore, their sequences can also be compared.

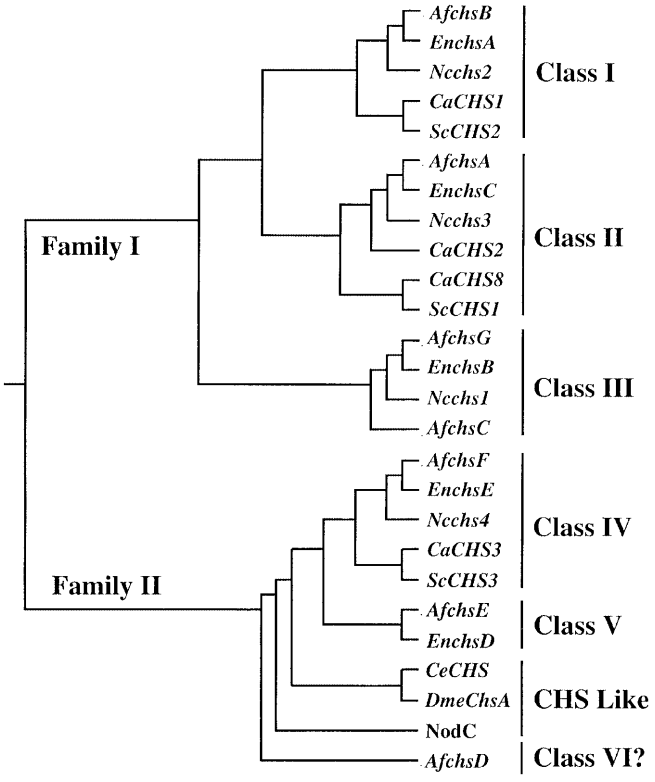

Fig. 2. Neighbour-joining tree of CS proteins from different organisms after Clustal W analysis (Thompson et al. 1994). The protein sequences compared include the conserved region shown in Fig. 1a plus the corresponding C-terminal extensions, although direct alignment of this domain gave very similar results (data not shown). The references for the original citations are given in Table 1, with the exception of the *C. albicans CaCHS8* sequence, which was obtained directly from the NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/Microb_blast/unfinishedgenome. html). *Af Aspergillus fumigatus*, *Ca C. albicans*, *Ce Caenorhabditis elegans*, *Dme Drosophila melanogaster*, *En A. nidulans*, *Nc Neurospora crassa*, *Sc S. cerevisiae*. *NodC* is from *Sinorhizobium meliloti*

The most obvious conclusion from the tree is that there are two well defined families of CSs. The upper part of Fig. 2 defines a family of genes that includes the *ScCHS1/2* and *CaCHS1/2* genes. However, the inclusion of fungal genes allows the subdivision of this family into three different classes: I, II and III. Apparently, classes I and II are present in yeast and filamentous fungi, but class III is exclusive to filamentous fungi. Several sequences isolated from other filamentous fungi

support the existence of class III *CHS* genes, but also their absence in yeasts.

The other family includes the homologues of the *ScCHS3* and *CaCHS3* genes. The proteins encoded by the genes included in this family share a high degree of similarity but are clearly distinct from the other family, a distinction that is also supported by analysis of their functions (Table 1). Nevertheless, while *S. cerevisiae* and *C. albicans*, both of them yeasts, contain only one of such genes, filamentous fungi contain two. Apparently, this would also be the case for other non-conventional yeasts (Valdivieso et al. 1999). Based on this difference, the separation of the members of this family into two classes was proposed. Class IV would be present in yeast and fungi, while class V would be exclusive to filamentous fungi. Over the past few years, several other *CHS* genes belonging to class V have been isolated and, surprisingly, all of them have been found to encode proteins of around 1,500 amino acids and contain a myosin motor-like domain in their amino-terminal part (Munro and Gow 2001). The only exception appears to be the *UmCHS6* gene (Xoconostle-Cazares et al. 1997), although a likely explanation for this discrepancy is that it would be due to a sequencing error. In *Aspergillus fumigatus* (Mellado et al. 1996b) and *A. oryzae* (Chigira et al. 2002), a sixth class of *CHS* genes was described (see also Fig. 2). However, caution should be exercised when defining new *CHS* classes before any further experimental work has been carried out.

Another very important conclusion can also be drawn from this picture, since *CHS*-like genes from insects and nematodes cluster with classes IV and V, suggesting a

Table 1. Functional characteristic of chitin synthases (*CS*) from different organisms. *None* No function yet proposed

Gene	Mutant defect	Proposed function	References
Class I			
ScCHS1	Lysis at acidic pH	Repair function during cytokinesis	Cabib et al. (1992)
CaCHS2	Minor reduction in chitin levels	Accessory enzyme?	Gow et al. (1994)
CaCHS8	Minor reduction in chitin levels	None	Munro and Gow (2001)
AfchsA	No apparent defect	None	Munro and Gow (2001)
EnchsC	No apparent defect	Redundant function in spore formation	Fujiwara et al. (2000)
WdCHS2	No apparent defect	None	Wang et al. (2001)
UmCHS	Minor reduction in chitin levels	None	Gold and Kronstad (1994); Xoconostle-Cazares et al. (1996)
Class II			
ScCHS2	Defective in septum formation; growth impaired	Synthesizes chitin of primary septum	Shaw et al. (1991); Silverman et al. (1988)
CaCHS1	Unviable	Involved in primary septum and lateral cell wall integrity	Munro et al. (2001)
AfchsB	No apparent defect	None	Munro and Gow (2001)
EnchsA	Minor reduction in chitin synthesis; required for Spore formation	CS involved in conidiogenesis	Culp et al. (2000); Fujiwara et al. (2000); Motoyama et al. (1997)
Ncchs2	No apparent defect	None	Din and Yarden (1994)
UmCHS2	40% reduction in chitin; no growth defect	Similar to ScCHS2?	Gold and Kronstad (1994); Xoconostle-Cazares et al. (1996)
Class III			
AfchsC	No apparent defect	None	Mellado et al. (1996a)
AfchsG	Abnormal growth; no chitin defect	CS required for hyphal growth	Mellado et al. (1996a)
EnchsB	Abnormal growth; no chitin defect	CS required for hyphal growth	Borgia et al. (1996)
Ncchs1	Abnormal growth; no chitin defect	CS required for hyphal growth	Yarden and Yanofsky (1991)
WdCHS3	No apparent defect	None	Wang and Szanislo (2000)
Class IV			
ScCHS3	Very reduced chitin levels	Major CS in vivo; not essential	Valdivieso et al. (1991)
CaCHS3	Very reduced chitin levels	Major CS in vivo; not essential	Bulawa et al. (1995); Mio et al. (1996)
AfchsF	No apparent defect	None	Munro and Gow (2001)
EnchsE	35% reduction in chitin	Involved in bulk chitin synthesis	Specht et al. (1996)
Ncchs4	Significant reduction in chitin	Major CS in vivo in some conditions	Din et al. (1996)
UmCHS5	50% chitin reduction; reduced virulence	Relevant CS in vivo	Xoconostle-Cazares et al. (1997)
WdCHS4	40% reduction in chitin	None	Wang et al. (1999)
Class V			
AfchsE	30% reduction in chitin; excessive hyphal swelling	Involved in bulk chitin synthesis	Aufauvre-Brown et al. (1997)
EnchsD	35% reduction in chitin; significant growth defect	Major CS in vivo	Specht et al. (1996); Horiuchi et al. (1999)
UmCHS6	70% reduction in chitin; required for virulence	Major CS in vivo	Xoconostle-Cazares et al. (1997)
WdCHS5	Required for virulence and growth at 37 °C	Major CS in vivo?	Liu et al. (2001)
Class VI			
AfchsD	20% reduction in chitin; no apparent defects	None	Mellado et al. (1996b)

common evolutionary origin for all CSs. In addition, the NodC gene from *Sinorhizobium meliloti* also shows significant similarity, suggesting a very ancient origin for these genes. Based on these data, it is possible to propose the main evolutionary trends in CSs. Their origin is very old, starting with a gene directly related to the second family and already present in bacteria. A very ancient gene duplication at the base of the fungal kingdom would have originated the two *CHS* families. The unique gene of family I also duplicated very early, so all fungi contain class I and II genes. Some time later, a further duplication led to the third class currently observed, although this third class appears to have emerged only in filamentous fungi. In certain specific cases, such as in *A. fumigatus*, further duplications occurred in the recent past, producing several isoenzymes in the same class. A similar situation can be found in family II in which, from an initial gene (class IV) present in all fungi, a duplication in the ancestor of filamentous fungi led to class V genes.

An interesting example of such evolutionary trends can be found in *Schizosaccharomyces pombe*, a relatively modern yeast, in which only one functional *CHS* gene has been described (*SpCHS1*; Arellano et al. 2000). This gene belongs to class I and only makes minute amounts of chitin during sporulation. Since vegetative cells do not contain appreciable amounts of chitin, it is not surprising that other *CHS* genes have been lost during evolution in this fission yeast.

Not only is this model coherent with the sequencing data, it is also supported by analysis of the functions of the different classes of CSs.

The function of CSs in fungi

Table 1 summarizes our current knowledge about the functions of CSs in different organisms. Although considerable efforts have been directed towards the characterization of the CS system in filamentous fungi, most of our knowledge comes from yeasts, especially *Saccharomyces cerevisiae*, where the power of genetic manipulation makes such study much easier. Therefore, we focus our discussion on yeast, leaving a broader discussion for the end of this section.

Early studies on *ScCHS1* indicated that it encodes the major in vitro CS, but that the activity of CSI does not have any in vivo function (Bulawa et al. 1986). Later work demonstrated that CSI is involved in cytokinesis and is responsible for repairing the chitin network after cell division. This activity counterbalances the role of chitinase in this process. The contribution of CSI to bulk chitin synthesis is negligible and the only phenotype observed in this mutant is a high degree of lysis in acidic media, where chitinase is more active (Cabib et al. 1989, 1992). The homologous gene in *C. albicans*, *CaCHS2*, could have a very similar function (Gow et al. 1994), although some indirect evidence suggests that it could also participate in the synthesis of chitin in lateral walls (Munro and Gow 2001).

ScCHS2 encodes a CS activity involved in the formation of the chitin disc that physically separates mother and daughter cells and constitutes the primary septum. Its function in cell biology is very important and it was originally described as being essential (Silverman et al. 1988). Further work revealed that this activity is only essential under certain specific growth conditions in *S. cerevisiae* (Bulawa and Osmond 1990). However, its *C. albicans* homologue, *CaCHS1*, was shown to be absolutely essential for cell survival (Munro et al. 2001). The very specific function of this activity demands a very tight regulatory mechanism that must be co-ordinated with the cell division cycle.

ScCHS3 encodes the CS activity responsible for more than 90% of cellular chitin (Valdivieso et al. 1991). Most of this chitin is localized in the ring that forms the bud scar, although a minor part is uniformly distributed throughout the cell wall (Shaw et al. 1991). In addition, this CS activity is responsible for synthesizing chitin at the base of the shmoo projection during mating and also during sporulation, when chitin is deacetylated to form the chitosan layer of ascospores (for a review, see Valdivieso et al. 1999). *chs3* mutant ascospores are immature due to the lack of chitosan and are more sensitive to damaging agents (Pammer et al. 1992). CSIII also forms an essential part of the salvage pathway response that ensures cell survival after cell wall damage (for a review, see Popolo et al. 2001). The picture seems to be very similar in *C. albicans*, although only limited information is available. Despite the major contribution to chitin synthesis, CSIII activity is completely dispensable in *C. albicans* and *chs3* cells grow normally (Bulawa et al. 1995; Mio et al. 1996). *C. albicans chs3* mutants are virulent, despite the considerable reduction in total chitin (Mio et al. 1996).

The data on fungal CS are more fragmented and sometimes even contradictory and it is therefore very difficult to arrive at direct conclusions. Some of these problems arose owing to the difficulty involved in obtaining double or triple mutants in filamentous fungi, a genetic tool that has been crucial to our understanding CS biology in yeast. The most important conclusions that can be drawn from our limited knowledge are that filamentous fungi have a family I CS isoenzyme directly involved in maintaining cell wall integrity, while they have another, from family II, that is more involved in chitin synthesis (see Table 1). This would be similar to what happens in yeast, where this role is attributed to class II and class IV enzymes respectively. However, the greater diversity of CSs in filamentous fungi means that these specific roles have been assumed by class III and class V enzymes, which are present only in these fungi and which are probably evolved from their yeast homologues. Very little is known about the myosin domain found in most fungal class V CSs, although some authors have speculated about the possibility of its use for the intracellular transport of this CS (Horiuchi et al. 1999).

The exact reasons for some filamentous fungi containing a higher number of *CHS* genes (see previous

section) remain unclear. However, these fungi have more complex differentiation patterns than yeast and therefore it would not be surprising that the higher diversity of CSs could be due to the specialization of each CS in single differentiation processes, which could be mediated by temporal or spatial restrictions.

The regulation of CSs

The variety of CSs in fungi, together with their specific function in the cell cycle, strongly suggests the existence of specific regulatory mechanisms for each of them. Again, our knowledge in this area comes from the yeast *Saccharomyces*, because what we know about filamentous fungi is several years behind our current knowledge about yeast. In this section, we describe the specific mechanisms involved in the regulation of each CS in *S. cerevisiae*, comparing them with the current limited knowledge derived from other fungal systems.

Regulation of CSI

CSI activity was originally described as zymogenic. However, no direct evidence for proteolytic processing of this activity in vivo has been demonstrated experimentally. The catalytic subunit of CSI is Chs1p, which is encoded by the *ScCHS1* gene (Bulawa et al. 1986). *ScCHS1* is expressed at high levels during vegetative growth, although it must be under transcriptional regulation because its expression increases strongly both during mating (Appeltauer and Achstetter 1989) and after activation of the salvage pathway (Valdivieso et al. 2000). We do not know the physiological relevance of this regulation, since absence of this activity does not produce any apparent phenotype under these biological conditions (Bulawa et al. 1986; Santos et al. 1997). The expression of the *CaCHS2* homologue is also induced shortly after the induction of hyphal formation (Chen-Wu et al. 1992; Munro et al. 1998).

Chs1p is fairly stable inside the cells (Choi et al. 1994a) and its levels do not change significantly during the cell cycle (Ziman et al. 1996). No genes directly involved in the control of CSI activity have been described, but the current model for this activity suggests that Chs1p would be mobilized from an internal pool of specialized vesicles, the chitosomes, from where it would be activated and sorted by an unknown mechanism (Ziman et al. 1996). The possibility of proteolytic regulation of this CS currently remains open.

Regulation of CSII

This activity, whose catalytic subunit is encoded by the *ScCHS2* gene, was originally described as zymogenic, but again there is no direct evidence for this type of regulation in vivo. *ScCHS2* expression is cell cycle-regulated (Igual et al. 1996) and, in consequence, CSII activity peaks just before cytokinesis (Choi et al. 1994a). Expression of this gene is strongly reduced during mating and sporulation, two conditions in which no primary septum is formed (Choi et al. 1994a). Our current model for this activity suggests that it is transported to the septum site by the general secretory pathway, where it acts in the formation of the primary septum (Shaw et al. 1991). Its function depends directly on the formation of the acto-myosin ring (Schmidt et al. 2002). CSII is inactivated by proteolytic processing in the vacuole after endocytosis. This inactivation is dependent on the general endocytic pathways and on Pep4 protease (Chuang and Schekman 1996). The homologous *CaCHS1* gene is expressed at low but constant levels, but no further insight into this regulation has been gained so far (Munro et al. 1998).

Regulation of CSIII

The existence of CSIII activity was demonstrated in 1990 (Bulawa and Osmond 1990), but its actual characterization began in 1994 (Choi and Cabib 1994; Choi et al. 1994b), although its activity was already known to depend on several genes (see above for a historical perspective). The catalytic subunit of this activity is Chs3p (Valdivieso et al. 1991), but the products of the *CHS4*, *CHS5*, *CHS6* and *CHS7* genes are required for functional CSIII activity (for a review, see Valdivieso et al. 1999), although they do not code for CS proteins.

Chs3p levels remain fairly constant inside the cell, mainly because of the absence of transcriptional regulation during the cell cycle, but also because of a very extended protein half-life (Ziman et al. 1996). These results immediately suggested a complex posttranslational regulation, which was confirmed after observing that high levels of Chs3p do not produce any phenotype, either in vivo or in vitro (Cos et al. 1998).

The current model indicates that CSIII activity is regulated inside the cells by a specialized mechanism of vesicle sorting (Ziman et al. 1996), coupled with a pathway based on endocytic recycling (Chuang and Schekman 1996; Holthuis et al. 1998). In this model, Chs3p protein is maintained inside specialized vesicles called chitosomes (TGN/early endosome vesicles) and is transported to the specific sites of function (Valdivia et al. 2002), where it becomes activated. Inactivation occurs via endocytosis. However, the protein is not degraded but instead is maintained inside the chitosomes (Chuang and Schekman 1996; Valdivia et al. 2002). The involvement of other *S. cerevisiae* Chs proteins in this regulation is discussed below.

Metabolic regulation of chitin synthesis in *S. cerevisiae*

Very recently, an unexpected mode of chitin synthesis regulation in yeast was proposed. This mode of regulation would be based on the availability of the substrate

for CSs: UDP-*N*-acetyl-glucosamine. The expression of the *GFA1* gene, which is directly involved in glucosamine metabolism, is increased when more chitin is required for cell growth, while alterations in the expression of this gene produce a direct effect on the rate of chitin synthesis (Lagorce et al. 2002). To date however, the specific effect of this regulation on each CS activity remains unknown.

This type of regulation might be of crucial importance in the responses mediated by the compensatory mechanism in yeast (Lagorce et al. 2002; Popolo et al. 2001), a situation in which chitin synthesis is increased, but in which conventional regulatory mechanisms do not appear to play any relevant role (Garcia-Rodriguez et al. 2000).

The role of other *chs* genes in yeast

Much of the story concerning CSIII regulation was elucidated through the characterization of the other *CHS* genes in *S. cerevisiae*, whose specific functions are summarized in Table 2 and are described below.

CHS7

This encodes an integral membrane protein that is located at the ER (Trilla et al. 1999). Chs7p acts as a specific chaperone for Chs3p, allowing its sorting from the ER (Fig. 3). Chs7p appears to remain in the ER; and hence its role was assumed to be similar to that of the Shr3p protein in the transport of several amino acid permeases. In the absence of this protein, Chs3p accumulates in the ER, producing an inactive protein both in vivo and in vitro (Trilla et al. 1999).

Chs7p appears to be present in limited amounts in the cell, since overexpression of Chs3p leads to a strong accumulation of this protein in the ER that can be relieved by increasing the intracellular amounts of Chs7p. This joint overexpression produces significantly higher amounts of CSIII, suggesting that the transcriptional regulation of *CHS7* could be an important mechanism in the regulation of chitin synthesis in vivo. Consistent with this notion, *CHS7* expression is increased under several experimental conditions (Trilla et al. 1999).

CHS6

CHS6 was initially described as *CSD3* (Bulawa 1992) and was shown to be required for chitin synthesis in vivo but not for CSIII activity in vitro (Bulawa 1993). This observation would place the participation of Chs6p in a late step in the control of CSIII activity. Today, we know that it is also involved in CSIII activity, since mutants have some defects in this activity (our unpublished data). This had previously gone unnoticed, probably for technical reasons. It is a Golgi protein required for the anterograde transport of Chs3p to the membrane (Fig. 3; Ziman et al. 1998). Very recently, it was shown to be involved in the correct transport of the TGN/early endosome vesicles containing Chs3p to the plasma membrane (Valdivia et al. 2002). Its function and localization seem to be very similar to those of Chs5p, although this protein is required for proper sporulation but not for mating (our unpublished data), exactly the opposite of what was reported for Chs5p (Santos et al. 1997).

S. cerevisiae contains a close homologue of Chs6p named YKR027p (43% identity). However, this gene has no apparent function in the control of chitin synthesis either in vivo or in vitro (our unpublished data).

CHS5

CHS5 was identified from a mutant isolated as being resistant to calcofluor white (Roncero et al. 1988). The

Table 2. Relevant characteristics of proteins involved in CSIII activity in *Saccharomyces cerevisiae*

Protein	Intracellular localization	Proposed function	Reference
Chs3p	Integral plasma membrane protein	Catalytic subunit of CSIII	Valdivieso et al. (1991)
Chs7p	Integral ER protein	Required for Chs3p exit from ER	Trilla et al. (1999)
Chs6p	Golgi protein	Required for Chs3p transport into PM from TGN/early endosomal vesicles[a]	Valdivia et al. (2002)
Chs5p	Golgi protein	Required for Chs3p transport into PM from TGN/early endosomal vesicles[a]	Santos and Snyder (1997); Valdivia et al. (2002)
Chs4p	Plasma membrane-associated protein	Probable direct activator of CSIII through its interaction with Chs3p; involved in Chs3p localization at the septum site	DeMarini et al. (1997), Ono et al. (2000); Trilla et al. (1997)
Bni4p	Septin-associated protein	Involved in anchoring the CSIII complex to the septin structure through direct interaction with Chs4p	DeMarini et al. (1997)
Shc1p	Plasma membrane-associated protein	Exclusively expressed during sporulation; probable direct activator of CSIII through interaction with Chs3p during spore formation	Sanz et al. (2002)

[a] Although the proposed function for Chs5p and Chs6p is the same, it remains to be tested whether both proteins are involved in exactly the same step

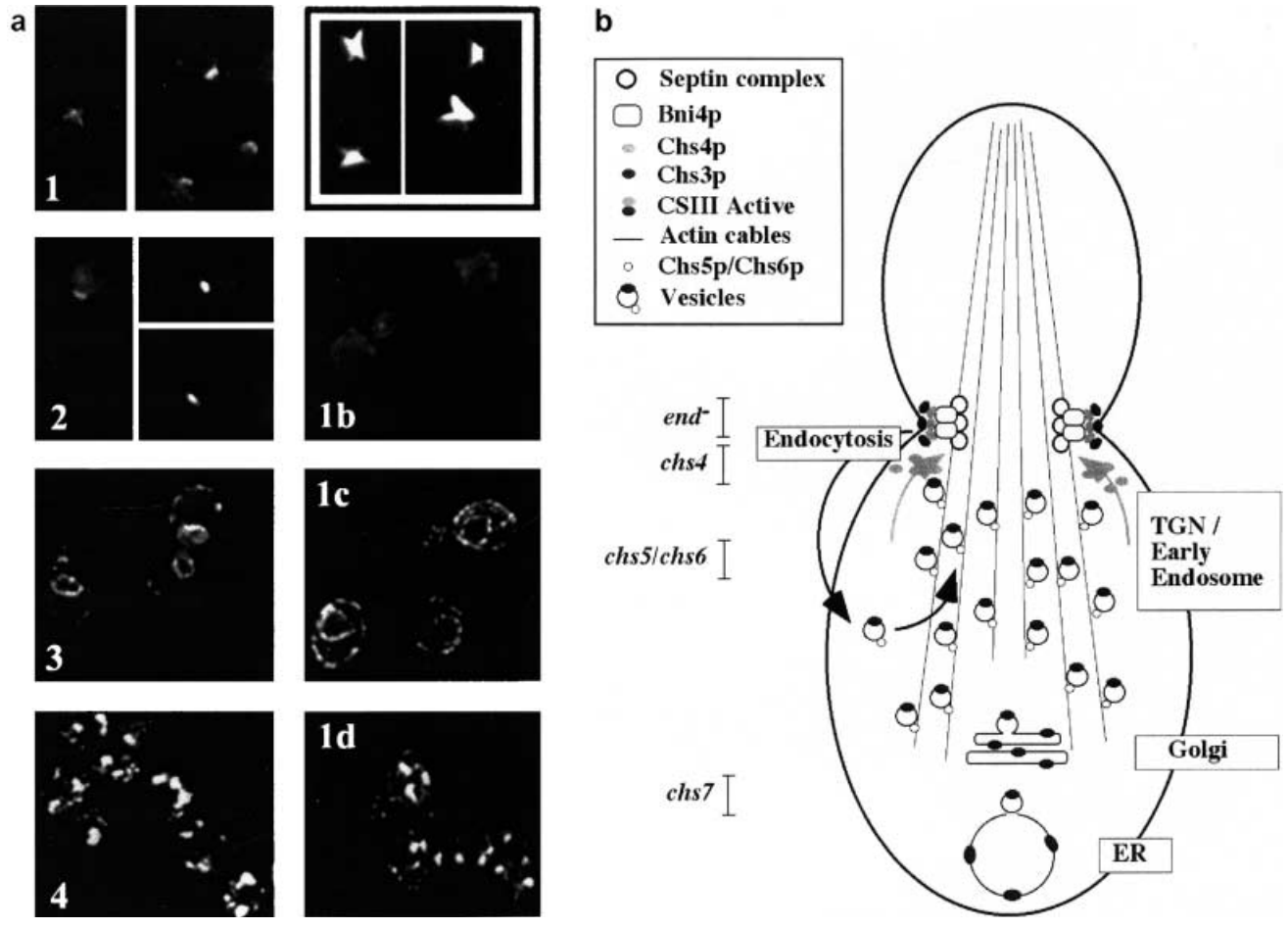

Fig. 3a, b. Schematic representation of Chs proteins in *Saccharomyces cerevisiae*. **a** Subcellular localization of Chs3p (*1*), Chs4p (*2*), Chs7p (*3*) and Chs5p (*4*) in wild-type strains and localization of Chs3p in *chs4* (*1b*), *chs7* (*1c*) and *chs5* (*1d*) mutant strains. The *insert* shows chitin localization in a wild-type strain after calcofluor white staining. Chs3p and Chs5p were localized by indirect immunofluorescence after protein tagging with the haemagglutanin epitope. Chs4p and Chs7p are visualized as green fluorescent protein-chimaeric proteins. Specific methods are described by Trilla et al. (1999). **b** Chs3p intracellular flux through the secretion machinery, from the ER compartment to the septum site. Brackets on the left indicate the subcellular localization where Chs3p transit is stopped in the absence of each protein

Chs5p protein was shown to be required for CSIII activity and also for chitin synthesis in vivo (Santos et al. 1997). It appears to be a Golgi protein required for the correct sorting of Chs3p inside the cell, since *chs5* mutants accumulate this protein in Golgi vesicles (Fig. 3; Santos and Snyder 1997). Its function seems to be specifically related to Chs3p transport during vegetative growth, although its participation in the transport of other membrane proteins cannot be excluded. It was also shown that Chs5p is not required during sporulation but is very important during mating, where it is required for proper cell fusion (Santos et al. 1997). Although its function in this process is not completely understood ,it appears to participate in the transport of certain specific mating proteins, such as Fus1p (Santos et al. 1997).

Together with Chs6p, Chs5p is required in the recycling of endocytic vesicles (Valdivia et al. 2002), although it is not known whether these two proteins participate in exactly the same step of Chs3p sorting or in two different steps.

This protein contains a fibronectin type III domain (Bateman and Chothia 1996) and shows a striking resemblance to animal neurofilaments, which is probably related to its function in vesicle trafficking.

CHS4

This was originally reported as *SKT5* (Kawamoto et al. 1992), a gene involved in cell wall synthesis during protoplast regeneration. However, the identity with *CHS4* remained unnoticed because of a significant sequencing error. The original *chs4* mutant was isolated due to its resistance to calcofluor white (cal^R2) and was shown to be required for CSIII and chitin synthesis (Bulawa 1993; Roncero et al. 1988). However, further characterization of the mutant indicated that it produces functional but highly zymogenic CSIII activity (Choi et al. 1994b; Trilla et al. 1997). Overexpression of this gene produces a significant increase in CSIII activity

in vitro (Ono et al. 2000; Trilla et al. 1997). Taken together, these results suggest that Chs4p could act as a direct activator of CSIII activity. The physical interaction between Chs4p and Chs3p would also support this notion (DeMarini et al. 1997; Ono et al. 2000).

CHS4 was also identified in a synthetic lethality screening with the Cdc12p septin and was shown to interact with Cdc10p septin through an anchor protein named Bni4p, which plays a more general role in the assembly of the septum machinery (DeMarini et al. 1997). Cells devoid of *BNI4* have normal amounts of chitin and CSIII activity (our unpublished data). However, in this mutant chitin is mislocalized, expanding to the daughter cell through an excessive leakage of Chs3p to that location (Sanz et al. 2002). The current model indicates that a complex containing Chs3p/Chs4p is positioned at the septum site through its interaction with the Bni4p/Septin complex (DeMarini et al. 1997; see Fig. 3 for a detailed scheme). However, to date, the mechanism of this interaction and whether it is directly related to the activation of CSIII activity remain to be elucidated.

The product of this gene is required for chitin synthesis during mating but not for proper sporulation, because *chs4* mutants form completely mature ascospores (Trilla et al. 1997). The reason for this is a very rapid destruction of Chs4p at the beginning of the sporulation process, even though transcription of this gene continues well into the process (Sanz et al. 2002). Not surprisingly, *S. cerevisiae* contains a *CHS4* homologue (Fig. 4) that is specifically induced during sporulation: the *SHC1* gene. This gene, which shows 45% identity with *CHS4*, is required for the synthesis of the ascospore's chitosan layer and is therefore required for spore maturation (Sanz et al. 2002). It is not expressed during vegetative growth but, when expressed from an inducible promoter, it is able to restore chitin synthesis in a *chs4* mutant. Therefore, both genes are functionally redundant but biologically compartmentalized through differential expression. However, although Shc1p can restore chitin synthesis, it cannot properly direct Chs3p to the septum site, producing a delocalized deposition of chitin (Sanz et al. 2002). It appears to have the same function as Chs4p in activating CSIII, but lacking the localization domain.

Chs4p, Shc1p and other proteins are characterized by the presence of multiple SEL1 domains, a type of domain presumably involved in protein–protein interactions (Grant and Greenwald 1996). However, while Chs4p has seven repeats, Shc1p has only four. It remains to be seen whether these three missing repeats are responsible for the interaction of Chs4p with Bni4p.

C. albicans contains a *CHS4* homologue that was shown to complement the chitin synthesis defect of a *S. cerevisiae chs4* mutant by restoring CSIII activity (Sudoh et al. 1999). However, its effect on chitin localization has not yet been tested.

The *S. cerevisiae* genome contains an additional and distant homologue of *CHS4*/*SHC1*, the YDL203 gene (Fig. 4), which is apparently not involved in the chitin synthesis process.

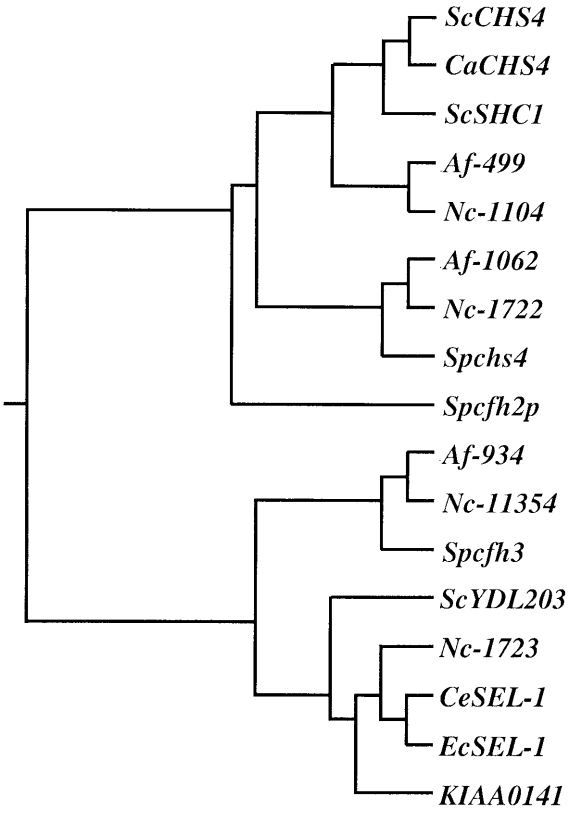

Fig. 4. Analysis of different *ScCHS4* homologues. Figure represents a neighbour-joining tree after Clustal W analysis (Thompson et al. 1994) of the complete protein sequences. Sequences were identified by BLAST analysis in different databases. *S. cerevisiae* sequences were obtained from SGD (http://genome-www.stanford.edu/Saccharomyces), *N. crassa* from http://www-genome.wi.mit.edu/annotation/fungi/neurospora and *Schizosaccharomyces pombe* from http://www.sanger.ac.uk/Projects/S_pombe. *A. nidulans*, *Candida albicans* and the remaining sequences were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov). Abbreviations identify each organism, together with the number of the contig containing the gene. Abbreviations are as in Fig. 2. *CeSEL-1* is from *Caenorhabditis elegans* (accession number U50828.1), *EcSEL-1* is from *Escherichia coli* (accession number D90704.1) and *KIAA0141* is from *Homo sapiens* (accession number NM 0147773.1)

CHS homologues in other fungi

Progress in the genome sequencing of other fungi currently allows the identification of putative *CHS* homologues in addition to the catalytic CS genes (see above). However, very little information has been obtained experimentally, although some work is under way.

Chs7p is unique in the *S. cerevisiae* genome. However, close homologues have been described in *C. albicans* (M. Sanz, unpublished data), *A. fumigatus* (C. Ortigosa, unpublished data), *Ashbya gossipyi* (NCBI, accession number AF195003) and *Neurospora crassa* (Whitehead Institute, Nc contig 3.322). The degree of

identity among all these genes is higher that 45%, which suggests similar functions. Nevertheless, *CaCHS7* cannot complement the *S. cerevisiae chs7* mutant (our unpublished data). It is likely that this gene would be present in most, if not all, fungi that have chitin in their cell walls. However, since the gene is involved exclusively in CSIII activity, it is not surprising that *Schizosaccharomyces pombe* and *Caenorhabditis elegans*, two organisms that do not have this activity and whose genomes have been completely sequenced, lack a *CHS7* homologue.

In addition to the YKR027 gene (see above), *Saccharomyces cerevisiae* contains several distantly related *CHS6* homologues whose function is not directly related to chitin synthesis. Surprisingly, database searches fail to identify any likely homologue of *CHS6* in other fungi, such as *C. albicans*, *Aspergillus fumigatus* or *N. crassa*. The level of protein identity found is lower than 24% (BLAST score 1×10^{-27}), suggesting that these genes could belong to the same family but are unlikely to be functional homologues. So far, the reason why other fungi do not have this gene remains a mystery.

Close homologues of the *ScCHS5* gene have been found in *C. albicans*, *A. fumigatus* and *N. crassa* (our unpublished data). The sequence similarity is sufficiently high (identity higher than 30%, BLAST score higher than 1×10^{-55}) to suggest a functional homology, although this remains to be tested experimentally.

The situation is more complex for *CHS4*, because this gene belongs to a huge family of genes, with representatives ranging from bacteria to humans. However, sequence comparisons indicate two very divergent gene groups (Fig. 4). While we have no clues as to the characterization of the lower group, the upper group is defined by the well characterized *ScCHS4* gene. Unfortunately, we do not have any experimental data on the fungal counterpart, but it should be noticed that *A. fumigatus* and *N. crassa*, like *Saccharomyces cerevisiae*, contain a pair of homologues. In *S. cerevisiae*, this pair is differentially regulated, indicating that fungal counterparts might also be subject to such differential regulation. *C. albicans* contains only one *CHS4*-like gene, possibly related to the lack of sporulation in this organism, which in turn leads to a lack of the differentially regulated homologue (see above). No clear evidence has been obtained with respect to the characterization of *Schizosaccharomyces pombe* homologues, but preliminary results indicate that these genes are not involved in cell wall synthesis or chitin synthesis regulation (M.H. Valdivieso, unpublished data).

Future prospects

One of the most interesting questions to be answered in the future is whether class IV and class V enzymes are subject to the same type of regulation. The answer to this question will necessarily have to address the functional characterization of the fungal class V enzymes, which could also help to explain the higher degree of diversity of CSs in filamentous fungi, compared with yeast. This work will likely be carried out in parallel with the characterization of some of the regulators of CS in fungi. At the same time, such efforts should help in the re-evaluation of this enzymatic activity as an antifungal target.

The other important question to be solved is the mechanism of activation/inactivation of CSIII in yeast and filamentous fungi. In this respect, we believe that the molecular characterization of the Chs4p function, in conjunction with the characterization of the TGN compartment where Chs3p is maintained, will be decisive in answering this issue.

Regarding family I CSs, the precise function of the different fungal isoenzymes remains to be characterized. It could be crucial to the understanding of the participation of some of these CSs in septum formation, a process believed to be essential for fungal growth. This field will require further progress in the characterization of ScChs2p and their likely filamentous fungal counterparts, the class III isoenzymes.

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References

Appeltauer U, Achstetter T (1989) Hormone-induced expression of the *CHS1* gene from *Saccharomyces cerevisiae*. Eur J Biochem 181:243–247

Arellano JM, Cartagena-Lirola H, Nasser-Hajibagheri MA, Duran A, Valdivieso MH (2000) Proper ascospore maturation requires the chs1+ chitin synthase gene in *Schizosaccharomyces pombe*. Mol Microbiol 35:79–89

Aufauvre-Brown A, Mellado E, Gow NA, Holden DW (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

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

Bateman A, Chothia C (1996) Fibronectin III domain in yeast detected by a hidden Markov model. Curr Biol 6:1544–1547

Borgia PT, Iartchouk N, Riggle PJ, Winter KR, Koltin Y, Bulawa CE (1996) The *chsB* gene of *Aspergillus nidulans* is necessary for normal hyphal growth and development. Fungal Genet Biol 20:193–203

Bulawa CE (1992) *CSD2*, *CSD3*, and *CSD4*, genes required for chitin synthesis in *Saccharomyces cerevisiae*: the *CSD2* gene product is related to chitin synthases and to developmentally regulated proteins in *Rhizobium* species and *Xenopus laevis*. Mol Cell Biol 12:1764–1776

Bulawa CE (1993) Genetics and molecular biology of chitin synthesis in fungi. Annu Rev Microbiol 47:505–534

Bulawa CE, Osmond BC (1990) Chitin synthase I and chitin synthase II are not required for chitin synthesis in vivo in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 87:7424–7428

Bulawa CE, Wasco W (1991) Chitin and nodulation. Nature 353:710

Bulawa CE, Slater M, Cabib E, Au-Young J, Sburlati A, Adair WLJ, Robbins PW (1986) The *S. cerevisiae* structural gene for chitin synthase is not required for chitin synthesis in vivo. Cell 46:213–225

Bulawa CE, Miller DW, Henry LK, Becker JM (1995) Attenuated virulence of chitin-deficient mutants of *Candida albicans*. Proc Natl Acad Sci USA 92:10570–10574

Cabib E, Roberts R, Bowers B (1982) Synthesis of the yeast cell wall and its regulation. Annu Rev Biochem 51:763–793

Cabib E, Sburlati A, Bowers B, Silverman SJ (1989) Chitin synthase 1, an auxiliary enzyme for chitin synthesis in *Saccharomyces cerevisiae*. J Cell Biol 108:1665–1672

Cabib E, Silverman SJ, Shaw JA (1992) Chitinase and chitin synthase 1: counterbalancing activities in cell separation of *Saccharomyces cerevisiae*. J Gen Microbiol 138:97–102

Cabib E, Shaw JA, Mol PC, Bowers B, Choi WJ (1996) Chitin biosynthesis and morphogenetic processes. In: Brambl R, Marzluf GA (eds) The Mycota. Biochemistry and molecular biology, vol 3. Springer, Berlin Heidelberg New York, pp 243–267

Chen-Wu JL, Zwicker J, Bowen AR, Robbins PW (1992) Expression of chitin synthase genes during yeast and hyphal growth phases of *Candida albicans*. Mol Microbiol 6:497–502

Chigira Y, Abe K, Gomi K, Nakajima T (2002) *chsZ*, a gene for a novel class of chitin synthase from *Aspergillus oryzae*. Curr Genet (in press)

Choi W, Cabib E (1994) The use of divalent cations and pH for the determination of specific yeast chitin synthases. Anal Biochem 219:368–372

Choi W, Santos B, Duran A, Cabib E (1994a) Are yeast chitin synthases regulated at the transcriptional or the posttranslational level? Mol Cell Biol 14:7685–7694

Choi W, Sburlati A, Cabib E (1994b) Chitin synthase 3 from yeast has zymogenic properties that depend on both the *CAL1* and *CAL3* genes. Proc Natl Acad Sci USA 91:4727–4730

Chuang JS, Schekman RW (1996) Differential trafficking and timed localization of two chitin synthase proteins, Chs2p and Chs3p. J Cell Biol 135:597–610

Cos T, Ford RA, Trilla JA, Duran A, Cabib E, Roncero C (1998) Molecular analysis of Chs3p participation in chitin synthase III activity. Eur J Biochem 256:419–426

Culp DW, Dodge CL, Miao Y, Li L, Sag-Ozkal D, Borgia PT (2000) The *chsA* gene from *Aspergillus nidulans* is necessary for maximal conidiation. FEMS Microbiol Lett 182:349–353

DeMarini DJ, Adams AEM, Fares H, De Virgilio C, Valle G, Chuang JS, Pringle JR (1997) A septin-based hierarchy of proteins required for localized deposition of chitin in the *Saccharomyces cerevisiae* cell wall. J Cell Biol 139:75–93

Din AB, Yarden O (1994) The *Neurospora crassa chs-2* gene encodes a non-essential chitin synthase. Microbiology 140:2189–2197

Din AB, Specht CA, Robbins PW, Yarden O (1996) *chs-4*, a class IV chitin synthase gene from *Neurospora crassa*. Mol Gen Genet 250:214–222

Fujiwara M, Ichinomiya M, Motoyama T, Horiuchi H, Ohta A, Takagi M (2000) Evidence that the *Aspergillus nidulans* Class I and Class II chitin synthase genes, *chsC* and *chsA*, share critical roles in hyphal wall integrity and conidiophore development. J Biochem 127:359–366

Gagou ME, Kapsetaki M, Turberg A, Kafetzopoulos D (2002) Stage-specific expression of the chitin synthase *DmeChSA* and *DmeChSB* genes during the onset of *Drosophila melanogaster*. Insect Biochem Mol Biol 32:141–146

Garcia-Rodriguez LJ, Trilla JA, Castro C, Valdivieso A, Duran A, Roncero C (2000) Characterization of the chitin biosynthesis process as a compensatory mechanism in the *fks1* mutant of *Saccharomyces cerevisiae*. FEBS Lett 478:84–88

Georgopapadakou N (2001) Update on antifungals targeted to the cell wall: focus on beta-1,3-glucan synthase inhibitors. Expert Opin Invest Drugs 10:269–280

Gold SE, Kronstad JW (1994) Disruption of two genes for chitin synthase in the phytopathogenic fungus *Ustilago maydis*. Mol Microbiol 11:897–902

Gow NAR, Robbins PW, Lester JW, Brown AJP, Fonzi WA, Chapman T, Kinsman OS (1994) A hyphal-specific chitin synthase gene (*CHS2*) is not essential for growth, dimorphism, or virulence of *Candida albicans*. Proc Natl Acad Sci USA 91:6216–6220

Grant B, Greenwald I (1996) The *Caenorhabditis elegans sel-1* gene, a negative regulator of *lin-12* and *glp-1*, encodes a predicted extracellular protein. Genetics 143:237–247

Holthuis JC, Nichols BJ, Pelham HR (1998) The syntaxin Tlg1p mediates trafficking of chitin synthase III to polarized growth sites in yeast. Mol Cell Biol 9:3383–3397

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

Igual JC, Johnson AL, Johnston LH (1996) Coordinated regulation of gene expression by the cell cycle transcription factor SWI4 and the protein kinase C MAP kinase pathway for yeast cell integrity. EMBO J 15:5001–5013

Kawamoto S, Nomura N, Ohno T (1992) Cloning and characterization of *SKT5*, a *Saccharomyces cerevisiae* gene that affects protoplast regeneration and resistance to killer toxin of *Kluyveromyces lactis*. J Ferment Bioeng 74:199–108

Lagorce A, Le Berre-Anton V, Aguilar-Uscanga B, Martin-Yken H, Dagkessamanskaia A, Francois J (2002) Involvement of *GFA1*, which encodes glutamine-fructose-6-phosphate amidotransferase, in the activation of the chitin synthesis pathway in response to cell-wall defects in *Saccharomyces cerevisiae*. Eur J Biochem 269:1697–1707

Liu H, Wang Z, Zheng L, Hauser M, Kauffman S, Becker JM, Szanislo PJ (2001) Relevance of chitin and chitin synthases to virulence in *Wangiella* (*Exophiala*) *dermatitidis*, a model melanized pathogen of humans. In: Muzarelli RAA (ed) Chitin enzymology 2001. Atec Edizioni, Rome, pp 463–472

Mellado E, Aufauvre BA, Gow, NAR, Holden DW (1996a) The *Aspergillus fumigatus chsC* and *chsG* genes encode class III chitin synthases with different functions. Mol Microbiol 20:667–679

Mellado E, Specht CA, Robbins PW, Holden DW (1996b) Cloning and characterization of *chsD*, a chitin synthase-like gene of *Aspergillus fumigatus*. FEMS Microbiol Lett 143:69–76

Mio T, Yabe T, Sudoh M, Satoh Y, Nakajima T, Arisawa M, Yamada-Okabe H (1996) Role of three chitin synthase genes in the growth of *Candida albicans*. J Bacteriol 178:2416–4219

Motoyama T, Fujiwara M, Kojima N, Horiuchi H, Ohta A, Takagi M (1997) The *Aspergillus nidulans* genes *chsA* and *chsD* encode chitin synthases which have redundant functions in conidia formation. Mol Gen Genet 253:520–528

Munro CA, Gow NAR (2001) Chitin synthesis in human pathogenic fungi. Med Mycol 39 [Suppl 1]:41–53

Munro CA, Schofield DA, Gooday GW, Gow NAR (1998) Regulation of chitin synthesis during dimorphic growth of *Candida albicans*. Microbiology 144:391–401

Munro CA, Winter K, Buchan A, Henry K, Becker JM, Brown AJ, Bulawa CE, Gow NAR (2001) Chs1 of Candida albicans is an essential chitin synthase required for synthesis at the septum and for cell integrity. Mol Microbiol 39:1414–1426

Ono N, Yabe T, Sudoh M, Nakajima T, Yamada-Okabe T, Arisawa M, Yamada-Okabe H (2000) The yeast Chs4p protein stimulates the trypsin-sensitive activity of chitin synthase 3 through an apparent protein–protein interaction. Microbiology 146:385–391

Orlean P (1997) Biogenesis of yeast wall and surface components. In: Pringle JR, Broach JR, Jones EW (eds) Cell cycle and cell biology. (The molecular and cellular biology of the yeast *Saccharomyces*, vol 3) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp 229–362

Pammer M, Briza P, Ellinger A, Schuster T, Stucka R, Bteintenbach M (1992) *DIT101* (*CSD2*, *CAL1*), a cell cycle-regulated yeast gene required for synthesis of chitin in cell walls and chitosan in spore walls. Yeast 9:1089–1099

Popolo L, Gualtieri T, Ragni E (2001) The yeast cell-wall salvage pathway. Med Mycol 39 [Suppl 1]:111–121

Roncero C, Valdivieso M, Ribas JC, Duran A (1988) Isolation and characterization of *Saccharomyces cerevisiae* mutants resistant to calcofluor white. J Bacteriol 170:1950–1954

Santos B, Snyder M (1997) Targeting of chitin synthase 3 to polarized growth sites in yeast requires Chs5p and Myo2p. J Cell Biol 136:95–110

Santos B, Duran A, Valdivieso MH (1997) *CHS5*, a gene involved in chitin synthesis and mating in *Saccharomyces cerevisiae*. Mol Cell Biol 17:2485–2496

Sanz M, Trilla JA, Duran A, Roncero C (2002) Control of chitin synthesis through Shc1p, a functional homologue of Chs4p specifically induced during sporulation. Mol Microbiol 43:1183–1195

Sburlati A, Cabib E (1986) Chitin synthase 2, a presumptive participant in septum formation in *Saccharomyces cerevisiae*. J Biol Chem 261:15147–15152

Schmidt M, Bowers B, Varma A, Roh DH, Cabib E (2002) In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. J Cell Sci 115:293–302

Semino CE, Specht CA, Raimondi A, Robbins PW (1996) Homologs of the *Xenopus* developmental gene DG42 are present in zebrafish and mouse and are involved in the synthesis of Nod-like chitin oligosaccharides during early embryogenesis. Proc Natl Acad Sci USA 93:4548–4553

Shaw JA, Mol PC, Bowers B, Silverman SJ, Valdivieso MH, Duran A, Cabib E (1991) The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. J Cell Biol 114:111–123

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

Smits GJ, Kapteyn JC, Ende H van den, Klis FM (1999) Cell wall dynamics in yeast. Curr Opin Microbiol 2:348–352

Smits GJ, Ende H van den, Klis FM (2001) Differential regulation of cell wall biogenesis during growth and development in yeast. Microbiology 147:781–784

Specht CA, Liu Y, Robbins PW, Bulawa CE, Iartchouk N, Winter KR, Riggle PJ, Rhodes JC, Dodge CL, Culp DW, Borgia PT (1996) The *chsD* and *chsE* genes of *Aspergillus nidulans* and their roles in chitin synthesis. Fungal Genet Biol 20:153–167

Sudoh M, Tatsuno K, Ono N, Chibana H, Yamada-Okabe H, Arisawa M (1999) The *Candida albicans CHS4* gene complements a *Saccharomyces cerevisiae skt5*/*chs4* mutation and is involved in chitin biosynthesis. Microbiology 145:1613–1622

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tkacz JS, DiDomenico B (2001) Antifungals: what's in the pipeline. Curr Opin Microbiol 4:540–545

Trilla JA, Cos T, Duran A, Roncero C (1997) Characterisation of *CHS4* (*CAL2*), a gene of *Saccharomyces cerevisiae* involved in chitin biosynthesis and allelic to *SKT5* and *CSD4*. Yeast 13:795–807

Trilla JA, Duran A, Roncero C (1999) Chs7p, a new protein involved in the control of protein export from the endoplasmic reticulum that is specifically engaged in the regulation of chitin synthesis in *Saccharomyces cerevisiae*. J Cell Biol 145:1153–1163

Valdivia RH, Baggot D, Chuang JS, Schekman R (2002) The yeast Clathrin adaptor protein complex 1 is required for the efficient retention of a subset of late Golgi membrane proteins. Dev Cell 2:283–294

Valdivieso MH, Mol PC, Shaw JA, Cabib E, Duran A (1991) *CAL1*, a gene required for activity of chitin synthase 3 in *Saccharomyces cerevisiae*. J Cell Biol 114:101–109

Valdivieso MH, Duran A, Roncero C (1999) Chitin synthases in yeast and fungi. In: Jolles P, Muzarelli RAA (eds) Chitin and chitinases. Birkhauser, Basel, pp 53–67

Valdivieso MH, Ferrario L, Vai M, Duran A, Popolo L (2000) Chitin synthesis in a *gas1* mutant of *Saccharomyces cerevisiae*. J Bacteriol 182:4752–4757

Veronico P, Gray LJ, Jones JT, Bazzicalupo P, Arbucci S, Cortese MR, Di Vito M, De Giorgi C (2001) Nematode chitin synthases: gene structure, expression and function in *Caenorhabditis elegans* and the plant parasitic nematode *Meloidogyne artiella*. Mol Genet Genom 266:28–34

Wang Z, Szanislo PJ (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

Wang Z, Zheng L, Hauser M, Becker JM, Szanislo PJ (1999) *WdCHS4*, a homolog of chitin synthase 3 in *Saccharomyces cerevisiae*, alone cannot support growth of *Wangiella* (*Exophiala*) *dermatitidis* at the temperature of infection. Infect Immun 67:6619–6630

Wang Z, Zheng L, Liu H, Wang Q, Hauser M, Kauffman S, Becker JM, Szanislo PJ (2001) WdCHS2p, a class I chitin synthase, together with WdCHS3p (Class III) contributes to virulence in *Wangiella* (*Exophiala*) *dermatitidis*. Infect Immun 69:7517–7526

Xoconostle-Cazares B, Leon-Ramirez C, Ruiz-Herrera J (1996) Two chitin synthase genes from *Ustilago maydis*. Microbiology 142:377–387

Xoconostle-Cazares B, Specht CA, Robbins PW, Liu Y, Leon C, Ruiz-Herrera J (1997) *Umchs5*, a gene coding for a class IV chitin synthase in *Ustilago maydis*. Fungal Genet Biol 22:199–208

Yarden O, Yanofsky C (1991) Chitin synthase 1 plays a major role in cell wall biogenesis in *Neurospora crassa*. Genes Dev 5:2420–2430

Ziman M, Chuang JS, Schekman RW (1996) Chs1p and Chs3p, two proteins involved in chitin synthesis, populate a compartment of the *Saccharomyces cerevisiae* endocytic pathway. Mol Biol Cell 7:1909–1919

Ziman M, Chuang JS, Tsung M, Hamamoto S, Schekman R (1998) Chs6p-dependent anterograde transport of Chs3p from the chitosome to the plasma membrane in *Saccharomyces cerevisiae*. Mol Biol Cell 9:1565–1576