The genetic complexity of chitin synthesis in fungi

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 polymers. 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).
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).