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Overview

Fungal cell wall biogenesis: building a dynamic interface with the environment

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Background

Developing an outer protective layer, namely the cell wall, is critical for growth and survival of the fungal cell in the diverse environments where fungi live. The eukaryotic nature of fungi makes the fungal cell a good model for addressing scientific questions related to the structure and function of this external layer in higher organisms. For decades, fungal cell wall studies relied mostly on the biochemical characterization of the major structural components, namely polysaccharides and proteins, and their biosynthesis. The introduction of gene cloning methodology allowed the identification and characterization of specific cell wall genes, which were cloned based on the information provided by their coded proteins. Genetic strategies also led to important breakthroughs with the isolation of cell wall mutants from species that were amenable to genetic analysis, especially the budding yeast *Saccharomyces cerevisiae*. These mutants were also the basis for identification of cell-wall-related genes. Molecular approaches thus became the obvious strategy to look into the essential question of cell wall biogenesis, which aims to understand the functions involved in designing the architecture and building the wall structure that serves as an interface of the cell with the environment.

The complexity of the cell wall means that its biogenesis demands a significant number of cellular activities that have to act in concert with the essential functions controlling cell growth and morphogenesis, since the wall determines cell shape. Genomic approaches have given an indication of the extent of genetic information required for cell construction in the budding yeast. About 20 % of *S. cerevisiae* genes control functions that are in some way related to cell wall biogenesis (De Groot *et al.*, 2001). The complexity of the protein components that can be found in the cell wall has also been addressed by subproteomic analyses (Pitarch *et al.*, 2002), which are opening new avenues for studies of the dynamics of the rigid, but not inert, layer that the cell wall represents. Genomic and proteomic strategies have opened up new possibilities for identification of specific, or related, cell wall genes and proteins, thus providing new, sometimes unexpected, possibilities for gaining insight into the formation of a complex structure that, being external to the cell membrane, is so critical for cell survival in most situations in nature.

Despite the high number of genes and proteins that have been described in relation to fungal cell wall biogenesis, we are far from having a complete picture of the set of processes that are operative in the fungal cell to create a wall structure, as is required for cell proliferation. Attempts to integrate the current information regarding cell wall can be found in several recent review articles (Cabib, 2004; Durán & Perez, 2004; Klis *et al.*, 2002; Valdivieso *et al.*, 2004). For the purpose of a clear presentation of major issues in current cell wall research, we can consider five main subjects (indicated below) that represent fields for exploration towards a comprehensive model of fungal cell wall development. This division is inevitably somewhat arbitrary, but it is useful to highlight the main avenues of cell wall studies. This issue of *Microbiology* includes a set of 23 original research papers contributing to our knowledge of fundamental aspects of fungal cell wall biogenesis. Although some of these papers deal with the cell wall of filamentous fungi, most of the studies reported are based on the systems that serve as models for fundamental research in yeast, namely the budding yeast *S. cerevisiae* or the opportunistic pathogenic fungus *Candida albicans*. The latter can give rise to either oval yeast cells or true hyphal forms, which involves significant remodelling of the cell wall, so to some extent it can represent an intermediate between yeasts and filamentous fungi. Both species, with a genome that has been sequenced and annotated, are very accessible for experimentation based on molecular approaches.

Biosynthesis of components and cell wall structure

A major polysaccharide, β-glucan, consisting of fractions with 1,3 or 1,6 linkages, together with a minor but very relevant chitin content, constitute the basic 'stones' of the cell wall in the form of a microfibrillar network that provides the rigidity of the yeast cell wall. The wealth of mannoproteins that are incorporated into this structure and the functions that they provide are far from being established. Issues such as the genes involved in the biosynthesis of polysaccharide components, including the secretion of the

enzyme complement required for this process to take place in an ordered manner, provide examples of questions that are currently being addressed. Such an array of proteins and structural polysaccharides maintains the integrity of the cell wall. Deficiencies in any of the functions are rarely lethal but they are expressed in terms of alterations that lead to a deficient wall that makes the cell more vulnerable.

Many of the cell wall proteins are GPI-anchored; the work of Freiman & Cormack (2004) provides insight into the molecular basis for sorting proteins of this type to localize either to the cell membrane or to the cell wall. The primary signal is provided by the amino acids immediately upstream of the site of GPI anchor addition (the ω site). With regard to the synthesis of GPI precursors, Orlean and coworkers show that CaSmp3p, which adds a fourth, α1,2-linked mannose to trimannosyl GPI precursors in *C. albicans*, is necessary for viability, thus providing an example of a critical gene that could represent a novel antifungal target. (Grimme *et al.*, 2004) The group of Klis and De Groot presents an *in silico* prediction analysis of the cell-surface GPI proteins and Pir proteins of the human pathogen *Candida glabrata*, complemented with some experimental data (Weig *et al.*, 2004). These results represent the first systematic analysis of the *C. glabrata* cell wall proteome.

Two papers deal with the role of mannoproteins that localize to the cell wall. Mrša and coworkers present evidence supporting the relevance of several mannoproteins, namely non-covalently bound, covalently bound or GPI-anchored cell wall proteins, for survival of the yeast cell under different conditions (Teparić *et al.*, 2004). Sentandreu and collaborators describe two alleles of the Pir1p of *C. albicans* that are expressed and play a role in the construction of the cell wall (Martínez *et al.*, 2004).

The question of glucan synthesis is dealt with by Machi *et al.* (2004), who explore the role of Rot1p, a highly conserved membrane protein, showing that deletion of the *ROT1* gene alters cell morphology, concomitant with reduction of glucan and increase of chitin content as well as impaired binding of GPI mannoproteins to the cell wall.

M. I. G. Roncero and coworkers revisit the question of the set of genes involved in chitin synthesis to describe the complex system that operates in the plant pathogen *Fusarium oxysporum* and its specificities compared to the thoroughly characterized system of *S. cerevisiae* (Martín-Udíroz *et al.*, 2004). From the morphological standpoint, basic ultrastructural features of the cell wall can still be discovered. In this regard, Coluccio & Neiman (2004) clearly demonstrate a new feature of the ultrastructure of the spore cell wall, namely interspore bridges, consisting of chitosan, and propose a role for these structure in the maintenance of heterozygous markers in a homothallic yeast population.

Cell wall assembly and remodelling

Fungal cells deprived of their cell wall can regenerate the structure under appropriate osmotic protection and nutritional conditions. However, when cells are growing under normal conditions, the development of the wall structure is based on the existing wall in the mother cells, which is extended and remodelled. The cell wall serves as a scaffold for the incorporation of new material into the growing points. The so far poorly understood role of many of the proteins that localize to the cell wall may be related to these processes, which must be essential for the enlargement of the wall structure.

Three papers in this issue shed new light on the functions involved in assembly of wall components. The group of Tanner and Strahl addresses the role of two proteins of the budding yeast, namely Scw4p and Scw10p, that belong to a family of glycosyl hydrolases and tranferases (Sestak *et al.*, 2004). By analysing the physiology of different mutants they conclude that Scw4p and Scw10p act as glucanases or transglucosidases in concert with other cell wall proteins to assure cell wall integrity. Schmitt and coworkers present evidence that Kre1p, a GPI-anchored *O*-glycoprotein required for 1,6-β-glucan formation, must play a structural role rather than performing an enzymic reaction (Breinig *et al.*, 2004). Finally, Lipke and collaborators provide an example of internal control of the correct development of the wall structure that occurs by cross-linking components to the network (Kipnis *et al.*, 2004). Using the GPI cell wall protein α-agglutinin, they show that the lack of Bet1p, a protein involved in ER–Golgi transition, which is required for the correct anchoring of the agglutinin, can cause severe defects in cell wall synthesis, although secretion of the agglutinin and other proteins continues.

Specialized processes in cell wall development: septum formation, polarized growth and spore wall formation

The specialization in cell wall development that takes place at certain regions represents an outstanding example of developmental regulation that again requires the concerted action of a number of genes and proteins. Septum formation occurs at the end of mitosis to achieve cell separation. The creation of a chitin ring is essential for progress to the formation of septum that will separate the cells. The group of Durán and Roncero has explored in detail the function of Bni4p, a protein related to chitin formation in *S. cerevisiae*. The evidence indicates that Bni4p is not only required for the assembly of the chitin ring but is also involved in septum architecture and the maintenance of neck integrity (Sanz *et al.*, 2004). Gow and coworkers have concentrated on the *C. albicans* homologue of the same gene. They show that in this case the lack of Bni4p function can alter chitin distribution throughout the cell wall, not only at the bud-neck region (Rowbottom *et al.*, 2004). With regard to the role of chitin synthesis, Schmidt (2004) provides evidence that the budding yeast cell can survive and divide in the absence of chitin synthesis, provided that cells are osmotically protected; the cells grow in chains with incompletely separated cytoplasms. Spontaneous mutations can suppress this phenotype and lead to chitin-free remedial septa.

The question of polarized growth is addressed by Momany *et al.* (2004), in a very elegant approach based on the development of monoclonal antibodies directed against epitopes of the *Aspergillus fumigatus* cell wall. The antibodies could recognize their determinants in intact cells of this fungus, which causes life-threatening infections. The labelling patterns enabled these workers to distinguish basal and apical domains in the cell wall and to conclude that the wall region flanking septa is different from other regions of the lateral wall. This approach should lead to the characterization of cell wall composition in local growing areas. Another specialized process in cell wall formation is addressed in the paper from the group of Nombela and Arroyo, which describes the role of Crr1p, the product of a gene expressed upon sporulation (Gómez-Esquer *et al.*, 2004). The protein, a putative transglycosidase, is located in the spore wall, and although it is not critically required for assembly of the spore wall itself, the lack of this function renders the spores more sensitive to stressful conditions.

Signal transduction and compensation for cell wall damage

One of the outstanding findings that have been made in the last few years is that fungal cells have developed mechanisms for adjusting to cell wall perturbation. Cell integrity is compromised by the many factors, either physical or chemical, that could alter the cell wall. The response that is activated to correct the alterations triggers a number of processes that require the expression of many genes related to the cell wall. The discovery of the 'cell integrity signalling pathway' (Cid *et al.*, 1995) was a clear demonstration that cell wall homeostasis is programmed and maintained for the cell to be protected against environmental stresses of many kinds. Several other signalling pathways are also related to this phenomenon and cross-talk with the cell integrity cascade, a process that is being actively explored and is far from being completely clarified. Several of the papers in this issue present new data contributing to our understanding of these central processes that assure cell integrity and link cell wall formation to cell wall growth and development.

Levin and coworkers document some specific features of Wsc1p of *S. cerevisiae*, an *O*-glycosylated membrane protein that acts as a sensor to activate the cell integrity pathway (Pkc1-controlled MAP kinase cascade) by interaction with the small G protein Rho1 (Vay *et al.*, 2004). Careful mapping of the cytoplasmic domains of the sensor identifies two important regions that play a role in regulation by phosphorylation mechanisms. Thorner and collaborators also address mechanistic details of activation of kinases relevant for cell integrity. They demonstrate that phosphorylation at PDK1 sites, and not at PDK2 sites, is indispensable for the activation of several other kinases, including Pkc1, that are involved in cell integrity (Roelants *et al.*, 2004).

By studying details of the activation of the two-component histidine kinase gene *CHK1* in *C. albicans*, Calderone and collaborators show that transcription of this gene is activated by several stresses, as well as by serum incubation (Li *et al.*, 2004). Ram *et al.* (2004) have looked at stress responses in filamentous fungi, and describe the relevance of the *gfa* gene encoding glutamine : fructose-6-phosphate amidotransferase. The transcription of this gene is activated by conditions that interfere with normal cell wall assembly and the general effect is to increase the levels of chitin in the cell wall. Schweizer and coworkers report on the role of some putative ATP : D-ribose-5-phosphate pyrophosphotransferases (*PRS1* to *PRS5* genes) in the construction of the *S. cerevisiae* cell wall and their involvement in the cell integrity pathway (Wang *et al.*, 2004).

The cell wall determines interaction with the environment

Cell protection by the complex wall structure is critical for survival, but many other components that are present in the cell wall determine various aspects of the interaction with the environment. Adhesion and recognition properties of the fungal cell reside in components of the cell wall that are also organized in the structure and are not independent of the other structural components. Three papers in this issue contribute novel information on the properties of the cell wall in relation to the external environment. The group of Nombela and Gil has characterized functional aspects of Ecm33p of *C. albicans*, a GPI protein homologous to least two other proteins found in the cell wall (Martinez-Lopez *et al.*, 2004). A functional Ecm33p is required for cell integrity and for an adequate formation of hyphae, and it is also critical for virulence of this pathogenic yeast. Meyer-Fernandes and coworkers have studied a phosphatase associated with the cell wall of the chromoblastomycosis agent *Fonsecaea pedrosi* (Kneipp *et al.*, 2004). They conclude that the presence of this enzyme in the cell wall is relevant for adhesion to mammalian cells.

Future prospects

Biogenesis of the fungal cell wall involves a highly complex set of processes that require many cell functions. Many processes have been characterized at the molecular level, by analysing the function of the many genes and proteins that are currently being identified in relation to cell wall functions. The original research papers in this issue provide a set of relevant examples of studies in this wide-ranging and active field.

Although the information that has so far have been obtained does not allow the formulation of a comprehensive model that could account for the integration of all the cell wall biogenetic processes described, we are coming closer to a situation where every mechanism can be linked to several others. These links represent the basis for further advancement towards the understanding of the cell wall as a unique structure that provides a dynamic interface with the environment. Large-scale approaches to the study of gene expression and the use of synthetic interaction networks

in relation to cell wall repair (García *et al.*, 2004; Lesage *et al.*, 2004) may provide the experimental basis for more comprehensive integration of the many observations related to fungal cell wall biogenesis.

The wealth of genetic information that is devoted to cell wall biogenesis must be a safeguard for the protection of the cell, so that failure in some of the processes can be compensated for by activation of other mechanisms to create the barrier that is required for cell stability. One of the main applications of fundamental knowledge of cell wall biogenesis in fungi is the identification of potential targets for new antifungals, which are especially needed for combating the life-threatening infections that several fungal species cause. In this regard, the arrival to clinical use of the first antifungal agent directed against the cell wall, namely caspofungin, a derivative of echinocandin, represents an important breakthrough that could be continued by exploitation of the fundamental information that is being derived from cell wall studies. Many other aspects, from fungal pathogenicity to biotechnological applications of fungi, should benefit from the continuing efforts to understand the fungal cell wall and its biogenesis.

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