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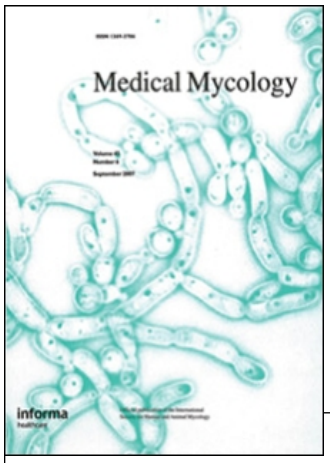

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Specific molecular features in the organization and biosynthesis of the cell wall of *Aspergillus fumigatus*

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The cell wall of *Aspergillus fumigatus* is composed of a branched β1,3 glucan covalently bound to chitin, β1,3, β1,4 glucans, and galactomannan, that is embedded in an amorphous cement composed of α1,3 glucan, galactomannan and polygalactosamin. The mycelial cell wall of *A. fumigatus* is very different from the yeast *Saccharomyces cerevisiae* cell wall, and in particular lacks β1,6 glucans and proteins covalently bound to cell wall polysaccharides. The differences in cell wall composition between the mould *A. fumigatus* and the yeast *S. cerevisiae* are also reflected at the genomic level where unique features have been identified in *A. fumigatus*. A single gene codes for the glucan synthase catalytic subumit; this finding has lead to the development of a RNAi methodology for the disruption of essential genes in *A. fumigatus*. In contrast to the glucan synthase, multiple genes have been found in the chitin synthase and the alpha glucan synthase families; in spite of homologous sequences, each gene in each family have very different function. Similarly homologous mannosyltransferase genes are found in yeast and moulds but they lead to the synthesis of very different N-mannan structures. This chemo-genomic comparative analysis has also suggested that GPI-anchored proteins do not have a role of linker in the three dimensional organization of the fungal cell wall.

Keywords cell wall, glucan, chitin, mannan, GPI

Introduction

A thorough analysis of the biochemical organization and biosynthesis of the cell wall of *Aspergillus fumigatus* is essential to obtain a good understanding of the growth of this filamentous fungus both *in vitro* and in its human host. All exchanges between the fungal cell and its environment rely upon a functional and permeable cell wall. In *A. fumigatus*, as in other pathogenic fungi, the cell wall is continously in contact with the host and acts as a sieve and a reservoir for molecules such as antigens and enzymes playing an active role during infection [1]. Moreover, it is essential to resist phagocytic killing [2,3] (Fig. 1).

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In conidial fungi such as *A. fumigatus*, cell wall remodeling occurs continously during morphogenesis (Fig. 1). The conidium has a double-layered cell wall with the outer layer being composed of melanin. During the first stages of germination (swelling), polysaccharide hydrolysis induces the softening and thinning of the original cell wall as well as *de novo* synthesis of a new electron lucent inner layer. The growing hyphae have a single monolayered cell wall. Cell wall biosynthesis appears to be a dynamic process which is essential and correlated with growth.

Polysaccharidic components of the cell wall are unique to fungi and consequently putative inhibitors of the biosynthetic pathways responsible for cell wall construction are unlikely to have secondary toxic effects in humans, as is the case for existing antifungal drugs such as amphotericin B. The recent launch of echinocandin antifungals that inhibit β1,3 glucan

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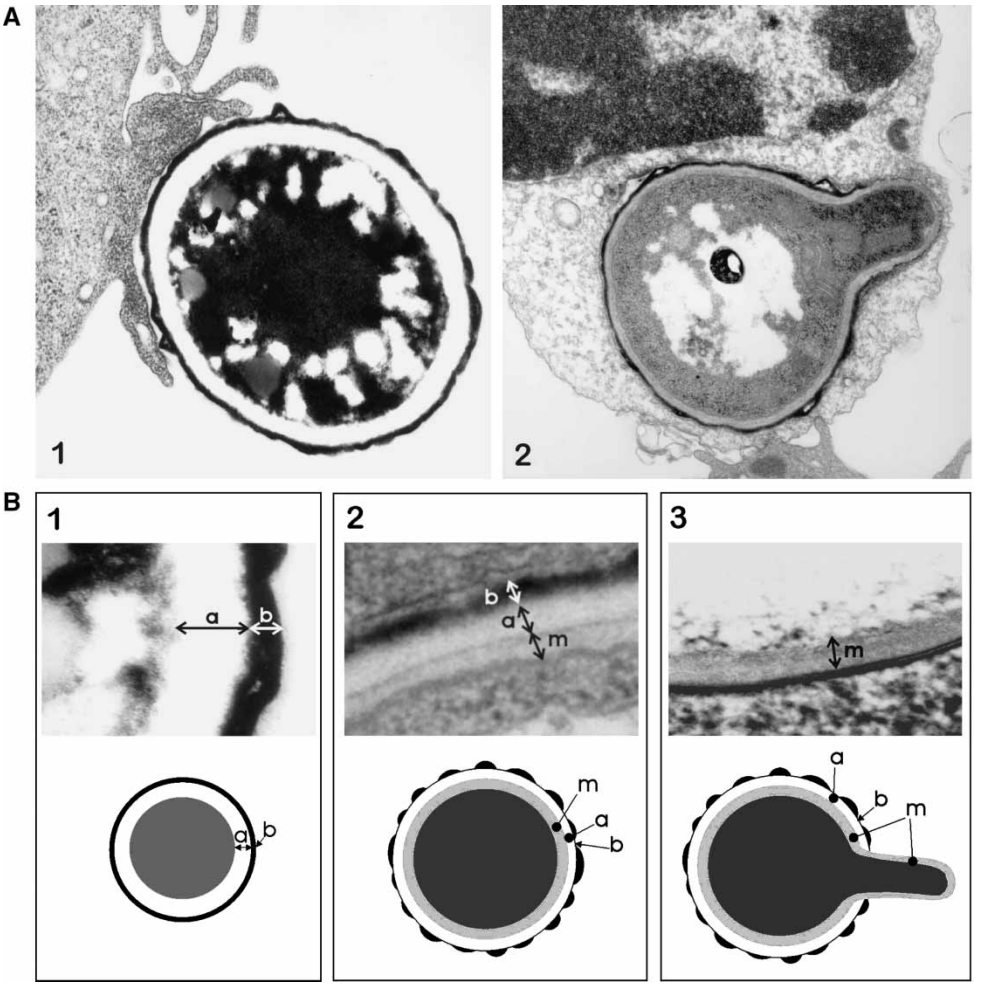

Fig. 1 (A). Conidial cell wall and host interactions. (1). Contact between macrophage pseudopods and conidium. (2). Germination of a conidium inside the alveolar macrophage of an immunocompromised mouse. (B). Cell wall remodeling during conidial germination. (1). Conidium cell wall with an electron lucent inner layer (a) and an outer melanin layer (b). (2). Stretching of the conidial cell wall and synthesis of a new cell wall layer (m) during conidial swelling. (3). Mycelial cell wall with a single layer (m) that originates from the de novo synthesized inner layer of the germinating conidium. The lower panel is a schematic representation of the upper figures.

synthase has validated the enormous potential of targeting the fungal cell wall [4].

These reasons are sufficient to investigate the polymer organization of the cell wall of *A. fumigatus* and the characterization of proteins/genes involved in the biosynthesis of the constitutive polymers of the cell wall. This paper reviews our current understanding of the structural organization of the cell wall and of the enzymes responsible for biosynthesis of the polysaccharides that make up the cell wall of *A. fumigatus*. This analysis takes into account recent genomic data emerging from the comparison of the *A. fumigatus* and *Saccharomyces cerevisiae* sequences.

Cell wall composition and organisation

The central core of the *A. fumigatus* cell wall is a branched β1,3, 1,6 glucan that is linked to chitin via a β1,4 linkage. This core, which accounts for about 30% of the total cell wall in *A. fumigatus,* is present in all fungi but possesses different additional bonds depending on the fungal species. In *A. fumigatus*, it is covalently bound to galactomannan and β1,3 β1,4 glucan [5]. Galactomannan is a branched structure composed of a linear α mannan with a repeating mannose oligosaccharide unit [6Manα1-2Manα1-2Manα1-2Manα1] and short chains of β(1,5) galactofuranose residues [6]. A linear β(1,3)/(1,4)-glucan, never previously described in fungi, was also found in *A. fumigatus* and represented 10% of total β-glucan. The linear β(1,3)/(1,4)-glucan had the following repeating unit [3Glcβ1-4Glcβ1]. This fibrillar β1,3 glucan complex is embedded in an alkali-soluble amorphous cement composed of α1,3 glucan and galactomannan [7].

Table 1 shows the composition of the cell walls of *A. fumigatus* and yeast. Qualitative and quantitative differences have been noted. For example, both fungi

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Table 1 Comparative cell wall composition in *A. fumigatus* and in *S. cerevisiae*

Cell wall polymers	*A. fumigatus*	*S. cerevisiae*
β1,3 glucan	+	+
Branched β1,3 glucan with β1,6 linkages	+	+
Chitin	+	+
Chitin-β1,3 glucan	+	+
α1,3 glucan	+	–
β1,6 glucan	–	+
β1,3, 1,4 glucan	+	–
Peptidomannan	–	+
Galactofuran	+	–
Galactomannan	+	–
Proteins	+	+
– unbound to polysaccharide	+	+
– covalently bound to polysaccharide	–	+

have chitin in their cell wall but the amount of chitin in the *A. fumigatus* mycelial cell wall is much higher than in yeast. This result is in accordance with the shape of the two fungi; simply put, more beams are necessary to support the structure of a tube such as a mycelium than that of a balloon such as an yeast. Accordingly, since chitin is thought to be responsible for maintaining the cell wall structure, higher amounts of chitin are expected in mycelial fungi. In dimorphic fungi, such as *Candida albicans*, hyphae contain more chitin than yeast [8].

In *S. cerevisiae*, β1,6 glucan and proteins bound to β glucans are present but the yeast does not have galactomannan, β(1,3) (1,4) glucan, or α1,3 glucan. A major feature in the cell wall of *A. fumigatus* is the lack of protein covalently bound to the polysaccharides [9]. In yeast, several studies have reported that numerous proteins originally anchored to the plasma membrane by a glycosylphosphatidyl inositol (GPI) anchor become later covalently linked to β1,3 glucans through a β1,6 glucan linker. The putative localization of the GPI-anchored proteins of *S. cerevisiae* in either the membrane or the cell wall has been investigated [10,11]. Caro *et al*. [10] based their results on the fact that plasma-membrane GPI proteins possess a dibasic residue motif that is absent in cell wall associated GPI proteins before their predicted GPI-attachment site. Hamada *et al*. [11] constructed fusion proteins of 40 C-terminal amino acids for each predicted GPI protein with a reporter protein and considered that only cell wall associated GPI proteins were released by treatment of the cell wall with laminarinase. Fusion proteins that were not released from the cell wall by the laminarinase treatment were exclusively classified as membrane bound GPI proteins. About half of the 50 GPI proteins that had localizations in concordance with the two studies were cell wall associated.

The search for genes coding for GPI-anchored proteins in *A. fumigatus* was undertaken amongst:

1. homologs to predicted GPI-anchored proteins of *S. cerevisiae* [10,11];
2. *A. nidulans* homologs predicted by "big-PI Fungal predictor" that is a GPI modification site prediction in fungi (http://mendel.imp.univie.ac.at/gpi/fungi_server.html) [12];
3. genes following the algorithm of De Groot *et al*. [13] identified in the TIGR database (http://www.tigr.org/tigr-scripts/proj/euk_manatee) by J. Wortmann [personnal communication].

This analysis has shown that bioinformatic algorithms predicting for GPI-anchored proteins in yeast are not always verified in *A. fumigatus*. For example, the absence of a dibasic residue before the GPI-attachment site is not an indication of a cell wall localization, nor is the algorithm of De Groot *et al*. [13] always verified for GPI anchoring of proteins in *A. fumigatus*.

Among the 81 GPI-proteins identified in *A. fumigatus*, only six families of GPI proteins in *A. fumigatus* were homologs of yeast GPI-proteins: *SPS2, GAS, DFG, PLB, CRH, YPS*. Five of these families were classified as membrane bound GPI proteins in yeast [10,11]. Genomic data are in agreement with a proteome study of GPI-anchored proteins of *A. fumigatus* [14]. Most of the *A. fumigatus* proteins (18 out of 22) identified in this study were homologs of membrane bound proteins in *S. cerevisiae*. In the *A. fumigatus* predicting protein, only one family was found that contained proteins with a putative cell-wall localization in *S. cerevisiae*. The five proteins of this family were orthologs of the *S. cerevisiae CRH* family. The proteins of this family have, however, sequence signatures suggesting a 1,3-β-glucanase activity [15], a finding that brings into question their covalent association with the cell wall. Four of the families mentioned above (*SPS2, CRH, GEL*/*GAS* and *DFG*) have been shown to be associated to yeast and mould cell wall construction, with some of them having enzymatic activities such as β1,3 glucanosyltransferases [15–18].

Very few proteins of *A. fumigatus* can remain strongly associated to the cell wall in absence of covalent linkages. One example of these proteins is PhoAp, an acid phosphatase of *A. fumigatus* [9]. Several convincing arguments show that the strong association between PhoAp and 1,3-β-glucan is not covalent:

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1. PhoAp is released from the cell wall of *A. fumigatus* by 1,3-β-glucanase in minute amounts, whereas the majority of PhoAp is extracted from the cell wall by the SDS/β-mercaptoethanol treatment or is secreted;
2. it was impossible to release all of the soluble proteins from the cell wall by detergent and reducing agent treatments. Indeed, incubation of the SDS/β-mercaptoethanol-treated cell wall sample in a buffer at 37°C overnight resulted in the passive release of soluble proteins including well-known secreted soluble antigens;
3. PhoAp is highly glycosylated with 12 putative N-glycosylation sites. As has been suggested for the yeast phosphatase, high amounts of mannosylation of PhoAp could facilitate strong interactions with the cell-wall β-1,3- glucans;
4. no β(1,6) glucan, to which are bound proteins in yeast, are present in the cell wall of *A. fumigatus;*
5. mass spectrometry analysis of PhoAp either after labeling of reducing sugars by 2-aminopyridine or trypsin digestion in presence of $H_2^{18}0$ did not show that the C-terminus of this peptide was covalently bound to an oligosaccharide [Bernard, unpublished data].

PhoAp appears then as a soluble protein tightly bound to the cell wall polysaccharide net by non-covalent bounds, that is released by the loosening of the net by the cell wall glycosylhydrolases. A similar situation could indeed occur for several so-called cell wall bound proteins in yeast, since the identification of the chemical linkages between the protein and the polysaccharide – which remains the ultimate proof of such covalent linkages – has been only assessed for the GPI-anchored protein TIP1 [19]. These data also show that the postulate 'release by β1,3 glucanase equals covalently linkage to the cell wall polysaccharide' is obviously not always true.

All the polysaccharide covalently bound proteins in yeast, such as *FLO, FIG* or *AGA* for *S. cerevisiae* or *ALS* and *EPA* in *Candida*, are involved in cell-to-cell adhesion or mating, or adhesion to host cell surfaces [20]. The covalent binding of proteins to polysaccharides is a way for the protein to remain at the surface of the cell wall where it has to bind directly to its ligand in order to fulfil its biological function (Fig. 2). The survey of the *A. fumigatus* genome and the biochemical investigation of the cell wall proteins of *A. fumigatus* have shown a lack of proteins covalently associated with the cell wall of *A. fumigatus*. GPI-anchored proteins in *A. fumigatus* seem to play only an enzymatic role in cell-wall biogenesis [17], without being covalently linked to the cell wall. This chemogenomic comparative analysis indicate that cell wall GPI-proteins do not have the role of linker between polysaccharides proposed for the establishment of the three-dimensional polysaccharide network of the yeast cell wall [21,22].

Biosynthesis of the major structural polysaccharides of the *A. fumigatus* cell wall

Cell wall polysaccharides are synthesized by polysaccharide synthases, which are transmembrane enzymes that use as a substrate intracellular nucleotide sugars. Although essential for cell wall construction, these enzymes remain insufficiently studied. Table 2 shows that the number of genes involved in the synthesis of cell wall polysaccharides is higher in *A. fumigatus* than in *S. cerevisiae*, suggesting that cell wall construction is more complex in filamentous fungi than in yeast. In *A. fumigatus*, four of the five gene families involved in cell wall polysaccharide synthesis have been studied and at least partially characterized. Non unique proteins of *A. fumigatus* were grouped into families taking into account both their partitions and clusters as obtained by the MCL algorithm [23,24]. Our knowledge on the synthesis of α and β glucan, galactomannan and chitin is reviewed below.

β glucan synthesis

β1,3 glucans of *A. fumigatus* are synthesized by a plasma membrane-bound glucan synthase complex, which uses UDP-glucose as a substrate and extrudes β1,3 glucan chains through the membrane into the periplasmic space. *FKS1*, a gene homologous to the *FKS* genes of *S. cerevisiae* which encode the putative catalytic subunit of the β1,3 glucan synthase, has been identified in *A. fumigatus*. Fks1p is an integral membrane protein with 16 transmembrane domains [25]. As in yeast, the presence of catalytic and regulatory subunits has been shown biochemically. However, no experiments using recombinant proteins have identified which one among the Rho proteins identified in the *A. fumigatus* genome is the regulatory subunit.

In *S. cerevisiae*, three *FK*S genes exist but only two are involved in β1,3 glucan synthesis [26]; the third one being a putative transporter [H. Bussey, personnal communication]. None of the single *FKS1* or *FKS2* disruptions were lethal. In contrast in *A. fumigatus*, *FKS1* is unique and the lack of transformants obtained by reverse genetics suggest it is an essential gene [25]. This gene is a unique tool to develop strategies that demonstrate the essentiality of a gene in an haploid

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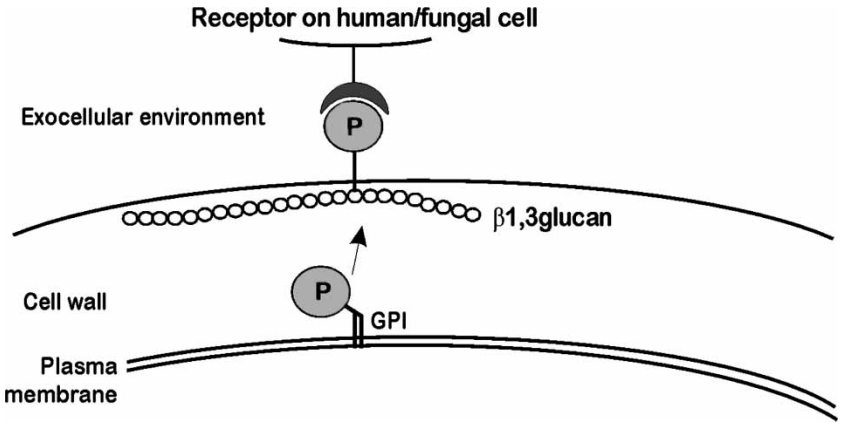

Fig. 2 A way for a GPI protein to fulfil its biological role in yeast. Protein (P) originally anchored to the plasma membrane by a GPI moiety, remains at the surface of the cell wall after binding to β1,3 glucans and can interact with other cell receptors.

fungus such as *A. fumigatus* for which unleaky inducible promoters have not yet been reported. The first strategy uses an artificially generated diploid strain that is haploidized after transformation [27]. Studies in our laboratory have indicated that this strategy cannot be used routinely since that transformation in a diploid background results in important uncontrolled deletions and inversions in the genome [Chabane, Henry, unpublished data].

Three genes involved in the molecular mechanism of RNA silencing have been identified and characterized in the fungus *Neurospora crassa* [28]. The first of these is *QDEI*, which is homologous to an RNA-dependent RNA polymerase. The second is *QDE2*, a homolog of *RDEI*, which is essential for dsRNA interference in *C. elegans*. *QDE3* is a member of the RecQ DNA helicase family. Homologs of *QDE1-3* have been identified in the TIGR *A. fumigatus* database with significant BLAST scores showing e values of 6×10^{-122}, 2×10^{-65} and 3×10^{-154}, respectively. The presence of these orthologs suggest that RNA interference (RNAi) should be functional in *A. fumigatus*. In RNAi, double stranded RNA induces the specific destruction of mRNA to which it is homologous [29]. RNAi was initiated by a hair-pin construct, where duplicate sequences of 500 bp of *FKS1* were cloned as inverted repeats separated by a 250-bp spacer. To control the expression of interfering RNA we used the glucoamylase promoter, which is induced in maltose medium and repressed in xylose medium. Protoplasts of *A. fumigatus* were transformed with the hygromycin B phosphotransferase marker circular interference construct in order to obtain ectopic integration in the genome. Using this strategy, we were able to show that *FKS1* is an essential gene in *A. fumigatus* [30]. Use of RNAi will allow easy examination of essential genes in *A. fumigatus*.

The genes coding for all enzymes listed in the KEGG web page (http://www.genome.jp/kegg/) for the synthesis of UDP-glucose, the substrate of β1,3glucan synthase, are present in the *A. fumigatus* genome [data not shown]. Accordingly, multiple alternative pathways can be used by this fungus to produce intracellularly the β-glucan synthase substrate.

Table 2 Synthases involved in the cell wall synthesis of *A. fumigatus* and *S. cerevisiae*

Enzyme activity	Gene	Substrate	Number of genes	
			A. fumigatus	*S. cerevisiae*
β1,3 glucan synthase	*FKS*	UDP-Glc	1	3[(a)]
Chitin synthase	*CHS*	UDP-GlcNAc	8	3
α1,3 glucan synthase	*AGS*	?	3	NA
Mannan synthase	*OCH1*	GDP-Man	3	1[(b)]
	Man Pol 1 complex[(c)]	″	3	3
Galactan synthase	?	?	NA	NA

NA: not appropriate.
[a]FKS3 is not associated to β1,3 glucan synthesis.
[b]*OCHI* has sequence homologies in yeast with another α1,6 mannosyltransferase *HOCI*.
[c]Mannan Pol complex1 contains 3 gene products (*MNN9, ANP1* and *VAN1*) in both fungi.

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Chitin synthesis

Chitin synthases (CHS) catalyse the polymerization of GlcNAc from UDP-GlcNAc. Chitin synthases of moulds have been split into six classes according to their aminoacid sequences (for only three in *S. cerevisiae*). Biochemical data have suggested that classes I–III are zymogenic, i.e. stimulated *in vitro* by trypsin, whereas classes IV to VI are non-zymogenic and directly responsible for the synthesis of the chitin cell wall. Classes III, V and VI have been only identified in filamentous fungi. Interestingly, mutants with the most altered phenotype result only from inactivation of *CHSE* and *G* genes belonging to class III and V, yielding a reduction in hyphal growth, periodic swellings along the length of hyphae and a block of conidiation partially restored by growth in presence of an osmotic stabilizer. A double *CHSE*/*CHSG* disruptant has been obtained and the phenotype of the double mutant is only additive [31]. The cell wall still contains chitin (half of the concentration of the parental strain) and the mutant still displays zymogenic and non-zymogenic chitin synthase activities. This result suggested an alternate regulation of *CHS* or the existence of undiscovered members of the *CHS* family. The latter hypothesis has been now confirmed by the analysis of the annotation of the *A. fumigatus* genome that has indicated the presence next to it of a duplicated copy of the *CHSE* and in the opposite direction (genes AFUM 72.m19439 and AFUM 72.m19440 in Fig. 3). Analysis of the common motifs among the eight chitin synthases of *A. fumigatus* using MEME/MAST programs [32] has shown two clusters of three (cluster 2) and four proteins (cluster 1) that are associated to chitin synthesis or chitin synthase activities respectively. These clusters have common motifs arranged in a different position in the sequence (Fig. 3). For example, the three common motifs of clusters 1 and 2 are very close to the C-terminus of proteins of cluster 2 and occupy a more central position in cluster 1. Only *CHSE* contains a single consensus motif that is homologous to kinesin or myosin motor-like domains, like its ortholog csmA of *A. nidulans* [33]. In contrast, the neighbouring duplicated gene does not contain any motif with similarity to kinesin and myosin.

The unique and essential pathway leading to the synthesis of UDP-GlcNAc exists also in yeast [34] *A. fumigatus*, D-glucosamine 6P and N-acetylglucosamine 6P that are key intermediates in this pathway can be produced directly from D-glucosamine and N-acetylglucosamine. It has never been investigated if the degradation of environmental chitin and chitosan easily attained in *A. fumigatus* which processes >15 chitinase and chitosanase genes could provide an intracellular source of glucosamine and N-acetylglucosamine to this fungus for further use for the synthesis of its own chitin.

α1,3 glucan synthesis

Three homologous genes *AGS* involved in α1,3 glucan synthesis have been identified. *AGS1* and *AGS2* were

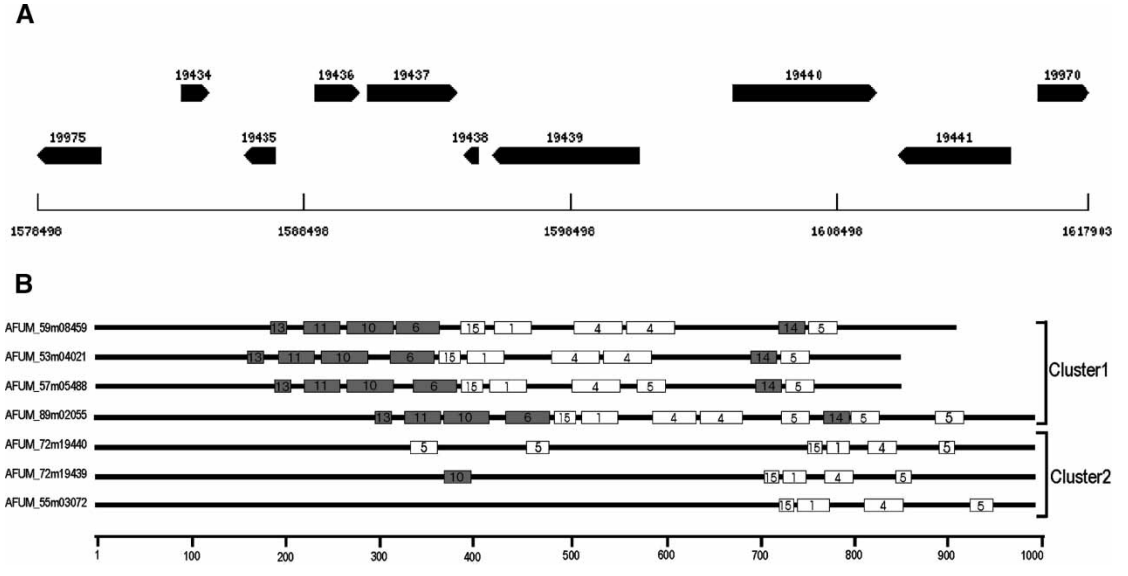

Fig. 3 (A). Duplication of the *CHSE* protein in the genome of *A. fumigatus* (see genes AFUM-72.m19439 and AFUM-72.m19440). (B). Organization of the two chitin synthase clusters of *A. fumigatus* as obtained by MEME/MAST programs [32]. Note that amino acid motifs 6, 11, 13, and 14 are only present in cluster 1, and that common motifs 1, 4, and 15 are in the same order but are not located in the same position in the genes of the two clusters.

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initially identified by screening a library with polymerase chain reaction products obtained after amplification of *A. fumigatus* DNA with degenerated primers, whereas the third was discovered in the genome. *AGS1–3* are around 8 kb long and each gene is characterized by two putative hydrolase and synthase domains separated by a single transmembrane fragment. Their sequences are very homologous to the *S. pombe* α1,3 glucan synthase genes but, in contrast to this yeast, all genes are expressed during vegetative growth and none of the *AGS* genes are essential in *A. fumigatus*. In spite of the high similarity between the three proteins, a partial reduction in α1,3 glucan was only seen in the cell wall of the *AGS1* mutant [7].

Galactomannan synthesis

Long *N*-mannans chains are usually considered as a 'coating' component of the yeast cell wall. While this is true in the case of the peptidomannans of yeasts, it is not true in *A. fumigatus* where mannan (as galactomannan) is an essential component of the cell wall and is bound covalently to the other polysaccharides. Although the mannan composition of the *A. fumigatus* cell wall is very different than the yeast mannans, a comparative genomic study has indicated that orthologs of most yeast mannosyltransferase genes can be found in the genome of *A. fumigatus*. Only one protein OCHI initiates synthesis of the long N-mannan chains in yeasts [35, 36]. A family of three OCHI homologs was found in the genome of *A. fumigatus*. The mannan polymerase complex 1 that is responsible of the elongation of the α1,6 mannan in yeast is composed of three genes, *MNN9*, *VAN1* and *ANP1*, that have unique orthologs in *A. fumigatus* although long stretches of linear α1,6 mannan are not present in *A. fumigatus*. These genes are analysed presently in *A. fumigatus* since the end products of this mannosyltransferase complex will be structurally different in both fungal species.

Mannan is linked to galactofuran. Genome analysis did not identify any gene involved in galactofuran biosynthesis as no homologs of the bacterial UDP-Gal pyranose mutase (37) were found in this eukaryotic genome.

Conclusion

The annotation of the sequence of *A. fumigatus* has been very helpful to complement our knowledge of the enzymes and regulators involved in cell wall synthesis in *A. fumigatus* and especially to identify new members in gene families. However, comparative genomics cannot give all the answers. To be efficient, such an approach requires a well established 'brother' genome with known functions for the encoded proteins. In the absence of reference genes, we were not able to identify any putative gene coding for protein involved in galactofuran biosynthesis, an *A. fumigatus* specific pathway, or enzymes responsible for the branching and cross linking of β1,3 glucan, a common structure essential for the cell wall of all yeasts and moulds.

Intraspecies and interspecies predicted proteome comparisons have so far been very successful. For example, analysis of the genome annotation of *A. fumigatus* has exhaustively identified all members of the chitin synthase, glucan synthase and mannosyltransferase families. Interspecies genomic comparisons are most potent when they are coupled to biochemical data. Such a combined approach has allowed us to revisit the role of proteins in fungal cell wall construction and correct a concept established erroneously in yeast. These chemical and genomic comparisons between species should prove very effective in the near future as a means to identify new pathways and edit concepts common to all fungi.

References

1 Bernard M, Latgé JP. *Aspergillus fumigatus* cell wall: composition and biosynthesis. *Med Mycol* 2001; **39**: 9–17.

2 Jahn B, Langfelder K, Schneider U, *et al*. PKSP-dependent reduction of phagolysosome fusion and intracellular kill of *Aspergillus fumigatus* conidia by human monocyte-derived macrophages. *Cell Microbiol* 2002; **4**: 793–803.

3 Philippe B, Ibrahim-Granet O, Prevost MC, *et al*. Killing of *Aspergillus fumigatus* by alveolar macrophages is mediated by reactive oxidant intermediates. *Infect Immun* 2003; **71**: 3034–3042.

4 Denning DW. Echinocandin antifungal drugs. *Lancet* 2003; **362**: 1142–1151.

5 Fontaine T, Simenel C, Dubreucq G, *et al*. Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *J Biol Chem* 2000; **275**: 27594–27607.

6 Latgé JP, Kobayashi H, Debeaupuis JP, *et al*. Chemical and immunological characterization of the galactomannan secreted by *Aspergillus fumigatus*. *Infect Immun* 1994; **62**: 5424–5433.

7 Beauvais A, Maubon D, Park S, *et al.* Two α(1–3) glucan synthases with different functions in *Aspergillus fumigatus. Appl Environm Microbiol* 2004: in press.

8 Chaffin WL, Lopez-Ribot JL, Casanova M, *et al*. Cell wall and secreted proteins of *Candida albicans*: identification, function, and expression. *Microbiol Mol Biol* 1998; **62**: 130–180.

9 Bernard M, Mouyna I, Dubreucq G, *et al*. Characterization of a cell-wall acid phosphatase (PhoAp) in *Aspergillus fumigatus*. *Microbiol* 2002; **148**: 2819–2829.

10 Caro LH, Tettelin H, Vossen JH, *et al*. In silicio identification of glycosyl-phosphatidylinositol-anchored plasma-membrane and cell wall proteins of *Saccharomyces cerevisiae*. *Yeast* 1997; **13**: 1477–1489.

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11 Hamada K, Fukuchi S, Arisawa M, *et al*. Screening for glycosylphosphatidylinositol (GPI)-dependent cell wall proteins in *Saccharomyces cerevisiae*. *Mol Gen Genet* 1998; **258**: 53–59.

12 Eisenhaber B, Schneider G, Wildpaner M, Eisenhaber F. A sensitive predictor for potential GPI lipid modification sites in fungal protein sequences and its application to genome-wide studies for *Aspergillus nidulans*, *Candida albicans*, *Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *J Mol Biol* 2004; **19**: 243–253.

13 De Groot PW, Hellingwer KJ, Klis FM. Genome-wide identification of fungal GPI proteins. *Yeast* 2003; **20**: 781–796.

14 Bruneau JM, Magnin T, Tagat E, *et al*. Proteome analysis of *Aspergillus fumigatus* identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis. *Electrophoresis* 2001; **22**: 2812–2823.

15 Rodriguez-Pena JM, Cid VJ, Arroyo J, Nombela C. A novel family of cell wall-related proteins regulated differently during the yeast life cycle. *Mol Cell Biol* 2000; **20**: 3245–3255.

16 Tougan T, Chiba Y, Kakihara Y, *et al*. Meu10 is required for spore wall maturation in *Schizosaccharomyces pombe*. *Genes Cells* 2002; **7**: 217–231.

17 Mouyna I, Fontaine T, Vai M, *et al*. GPI-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. *J Biol Chem* 2000; **275**: 14882–14889.

18 Kitagaki H, Wu H, Shimoi H, Ito K. Two homologous genes, DCW1 (YKL046c) and DFG5, are essential for cell growth and encode glycosylphosphatidylinositol (GPI)-anchored membrane proteins required for cell wall biogenesis in *Saccharomyces cerevisiae*. *Mol Microbiol* 2002; **46**: 1011–1022.

19 Fujii T, Shimoi H, Iimura Y. Structure of the glucan-binding sugar chain of Tip1p, a cell wall protein of *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 1999; **1427**: 133–144.

20 Verstrepen KJ, Reynolds TB, Fink GR. Origins of variation in the fungal cell surface. *Nat Rev Microbiol* 2004; **2**: 533–540.

21 Kapteyn JC, Van Den Ende H, Klis FM. The contribution of cell wall proteins to the organization of the yeast cell wall. *Biochim Biophys Acta* 1999; **1426**: 373–383.

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

23 van Dongen S. Markov Cluster algorithm: A cluster algorithm for graphs. http://micans.org/mcl/

24 Tekaia F, Blandin G, Malpertuy A, *et al*. Genomic exploration of the hemiascomycetous yeasts: 3. Methods and strategies used for sequence analysis and annotation. *FEBS Lett* 2000; **487**: 17–30.

25 Beauvais A, Bruneau JM, Mol PC, *et al*. Glucan synthase complex of *Aspergillus fumigatus*. *J Bact* 2001; **183**: 2273–2279.

26 Lesage G, Sdicu AM, Menard P, *et al*. Analysis of beta-1,3-glucan assembly in *Saccharomyces cerevisiae* using a synthetic interaction network and altered sensitivity to caspofungin. *Genetics* 2004; **167**: 35–49.

27 Firon A, Beauvais A, Latgé JP, *et al*. Characterization of essential genes by parasexual genetics in the human fungal pathogen *Aspergillus fumigatus*: impact of genomic rearrangements associated with electroporation of DNA. *Genetics* 2002; **161**: 1077–1087.

28 Cogoni C, Macino G. Isolation of quelling-defective (qde) mutants impaired in posttranscriptional transgene-induced gene silencing in *Neurospora crassa*. *Proc Natl Acad Sci USA* 1997; **94**: 10233–10238.

29 Hammond SM, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double stranded RNA. *Nat Rev* 2001; **2**: 110–119.

30 Mouyna I, Henry C, Doering TL, Latgé JP. Gene silencing with RNA interference in the human pathogenic fungus *Aspergillus fumigatus*. *FEMS Microbiol Lett* 2004; **237**: 317–324.

31 Mellado E, Dubreucq G, Mol P, *et al*. Cell wall biogenesis in a double chitin synthase mutant (chsG-/chsE-) of *Aspergillus fumigatus*. *Fungal Genet Biol* 2003; **38**: 98–109.

32 Bailey TL, Gribskov M. Combining evidence using p-values: application to sequence homology searches. *Bioinformatics* 1998; **14**: 48–54.

33 Horiuchi H, Fujiwara M, Yamashita S, *et al*. Proliferation of intrahyphal hyphae caused by disruption of *csm*A, which encodes a class V chitin synthase with a myosin motor-like domain in *Aspergillus nidulans*. *J Bacteriol* 1999; **181**: 3721–3729.

34 Lagorce A, Le Berre-Anton V, Aguilar-Uscanga B, *et al*. 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* 2002; **269**: 1697–1707.

35 Dean N. Asparagine-linked glycosylation in the yeast Golgi. *Biochem Biophys* 1999; **1426**: 309–322.

36 Jungmann J, Munro S. Multi-protein complexes in the cis Golgi of *Saccharomyces cerevisiae* with alpha alpha-1,6-mannosyltransferase activity. *EMBO J* 1998; **17**: 423–434.

37 Sanders DA, Staines AG, McMahon SA, *et al*. UDP-galactopyranose mutase has a novel structure and mechanism. *Nat Struct Biol* 2001; **8**: 858–863.