The structure and synthesis of the fungal cell wall

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Summary

The fungal cell wall is a dynamic structure that protects the cell from changes in osmotic pressure and other environmental stresses, while allowing the fungal cell to interact with its environment. The structure and biosynthesis of a fungal cell wall is unique to the fungi, and is therefore an excellent target for the development of anti-fungal drugs. The structure of the fungal cell wall and the drugs that target its biosynthesis are reviewed. Based on studies in a number of fungi, the cell wall has been shown to be primarily composed of chitin, glucans, mannans and glycoproteins. The biosynthesis of the various components of the fungal cell wall and the importance of the components in the formation of a functional cell wall, as revealed through mutational analyses, are discussed. There is strong evidence that the chitin, glucans and glycoproteins are covalently cross-linked together and that the cross-linking is a dynamic process that occurs extracellularly. ***BioEssays*** **28:799–808, 2006.**

Introduction

The fungal cell wall is a dynamic organelle that functions in a number of important processes. It must provide the cell with sufficient mechanical strength to withstand changes in osmotic pressure imposed by the environment. Concurrently, the cell wall must retain adequate plasticity to allow for cell growth, cell division and the formation of a myriad of cell types during the life cycle of the fungus. In addition to maintaining cell shape and integrity in the face of environmental stress, the wall allows the fungal cell to interact with its surroundings. The cell wall mediates the adhesion of cells to one another and the substratum, and serves as a signaling center to activate signal transduction pathways within the cell. Disruptions of cell wall structure have a profound effect on the growth and morphology of the fungal cell, often rendering it susceptible to lysis and death. Given the vital role that the cell wall plays in fungal physiology, the cell wall has long been considered an excellent target for anti-fungal agents.

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Fungal cell walls are structurally unique and differ significantly from the cellulose-based plant cell wall. Fungal cell walls are comprised of glycoproteins and polysaccharides, mainly glucan and chitin. Additional minor cell wall components are present and vary amongst species of fungi. The glycoproteins present in the cell wall are extensively modified with both N- and O-linked carbohydrates and, in many instances, contain a glycosylphosphatidylinositol (GPI) anchor as well. The glucan component is predominately beta-1,3-glucan, long linear chains of beta-1,3-linked glucose. Glucans having alternate linkages, such as beta-1,6-glucan, are found within some cell walls. Chitin is manufactured as chains of beta-1,4-linked N-acetylglucosamine residues and is typically less abundant than either the glycoprotein or glucan portions of the wall. The composition of the cell wall is subject to change and may vary within a single fungal isolate depending upon the conditions and stage of growth. The glycoprotein, glucan and chitin components are extensively cross-linked together to form a complex network, which forms the structural basis of the cell wall (Fig. 1).

The formation and remodeling of the cell wall involves numerous biosynthetic pathways and the concerted actions of hundreds of gene products within the fungal cell. Mutational, genomic and proteomic analyses of model fungal systems, such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Aspergillus fumigatus* and *Neurospora crassa*, are beginning to elucidate the role of various pathways and individual proteins in the establishment of the cell wall (Fig. 2).

The chitin component of the fungal cell wall

Chitin, a long linear homopolymer of beta-1,4-linked N-acetylglucosamine, is considered to be a relatively minor, yet structurally important, component of the fungal cell wall. Chitin accounts for only 1–2% of the yeast cell wall by dry weight,[1,2] whereas the cell walls of filamentous fungi, such as *Neurospora* and *Aspergillus*, are reported to contain 10–20% chitin.[3,4] In both yeasts and filamentous fungi, chitin microfibrils are formed from inter-chain hydrogen bonding. These crystalline polymers have an enormous tensile strength and significantly contribute to the overall integrity of the cell wall. When chitin synthesis is disrupted, the wall becomes disordered and the fungal cell becomes malformed and osmotically unstable.[5,6]

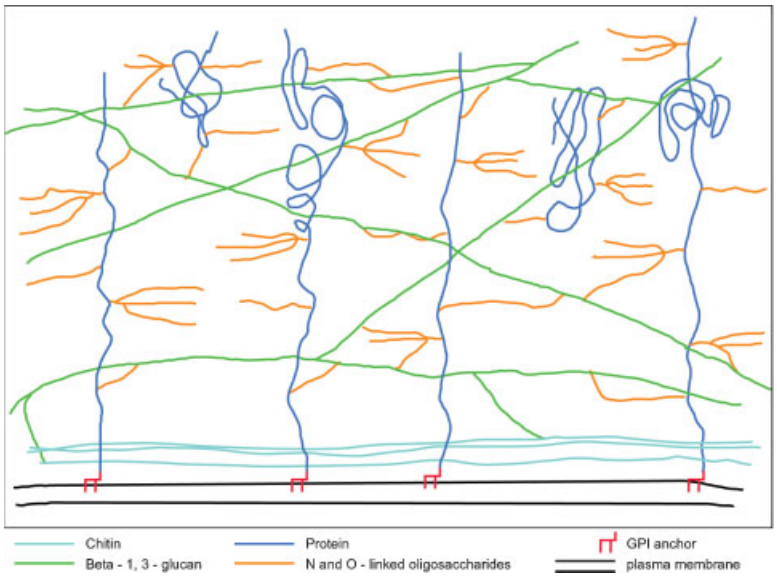

Figure 1. Representation of the fungal cell wall. The major components of the fungal cell wall are chitin, glucans and glycoproteins. Although species-specific variations exist, the cell wall components are thought to be arranged as shown. Most of the chitin is considered to be located near to the plasma membrane. The beta-1,3-glucan extends throughout the cell wall. The glycoproteins are extensively modified with N- and O-linked oligosaccharides. Many of the glycoproteins have GPI anchors, which tether them to the plasma membrane while other glycoproteins are secreted into the cell wall space. The proteins, glucans and chitin components are integrated into the wall by cross-linking the chitin, glucans, protein-associated oligosaccharides and GPI anchors together.

The synthesis of chitin is mediated by chitin synthase, an integral membrane enzyme that catalyzes the transfer of N-acetylglucosamine from uridine diphosphate (UDP)-N-acetylglucosamine to a growing chitin chain. The elongation of the chitin polymers occurs via vectorial synthesis, so that the nascent chains are extruded through the plasma membrane as they are made. Hydrogen bonding between the newly formed polymers of chitin results in microfibril formation and subsequent crystallization of chitin in the extracellular space immediately adjacent to the plasma membrane. This process of chitin synthesis primarily occurs at sites of active growth and cell wall remodeling. For yeasts, this includes areas such as the bud tip during polarized growth and the bud neck during cytokinesis. In filamentous fungi, localized areas of cell wall synthesis occur at the hyphal apex, or growing tip.

The specific roles of the chitin synthases of several fungi have been studied using both genetic and biochemical analyses. *Saccharomyces cerevisiae* has three chitin synthases, Chs1p, Chs2p and Chs3p.[7] Chs1p functions in cell wall repair, replenishing chitin polymers lost during cytokinesis. Chs2p is required for the formation of the primary septum within the dividing yeast cell.[8,9] The Chs3p chitin synthase is responsible for generating approximately 80–90% of the total cellular chitin.[10,11] This includes the chitin ring formed during bud emergence, as well as the chitin that becomes covalently linked to the beta-1,3-glucan fraction of the cell wall.[8,12] Mutants affected in the Chs3p chitin synthase have vastly reduced chitin levels and rates of growth, accompanied by defects in cell wall integrity. The simultaneous deletion of all three genes results in a lethal phenotype, demonstrating that chitin is an indispensable component of the cell wall of *S. cerevisiae*.[8,13]

A. fumigatus has seven chitin synthase-encoding genes designated as *CHSA* through *CHSF*. The genes encoding all seven chitin synthases of *A. fumigatus* have been cloned and disrupted. Null mutants for the *CHSA, CHSB, CHSC* and *CHSF* genes have no apparent phenotypic defect.[15,16] Disruption of the *CHSD* gene results in a 20% reduction in the production of chitin, yet the mutants are morphologically indistinguishable from wild-type cells.[17] *CHSG* mutants exhibit no apparent deficiency in the synthesis of chitin, but do have altered hyphal growth patterns.[16] The *CHSE* gene product appears to be the most critical for cell wall biosynthesis. *CHSE* null mutants experience a 30% reduction in total chitin synthesis, which results in excessive hyphal swelling and alterations in conidiation. The ChsE enzyme is proposed to be involved in the majority of general, bulk chitin synthesis.[18]

A similar situation exists in *N. crassa*, where four specific chitin synthases have been identified and three additional isoenzymes are predicted to exist based upon an analysis of its genome.[19–23] Those chitin synthases that have been identified and characterized are encoded by the *chs1, chs2, chs3* and *chs4* genes. Mutants for each of these genes have been isolated and the genes differ with respect to their importance for morphology. Null mutations within *chs1* result in only a modest reduction in the overall levels of chitin, yet yield mutants that grow slowly and produce swollen, bulbous hyphae.[19] Disruptions in *chs2* result in isolates that have normal gross morphologies and levels of cell wall chitin.[20] The *chs4* gene is thought to function in cell wall biosynthesis under environmental stress conditions.[21] The *chs3* gene of *N. crassa* is essential for survival, suggesting that the CHS3

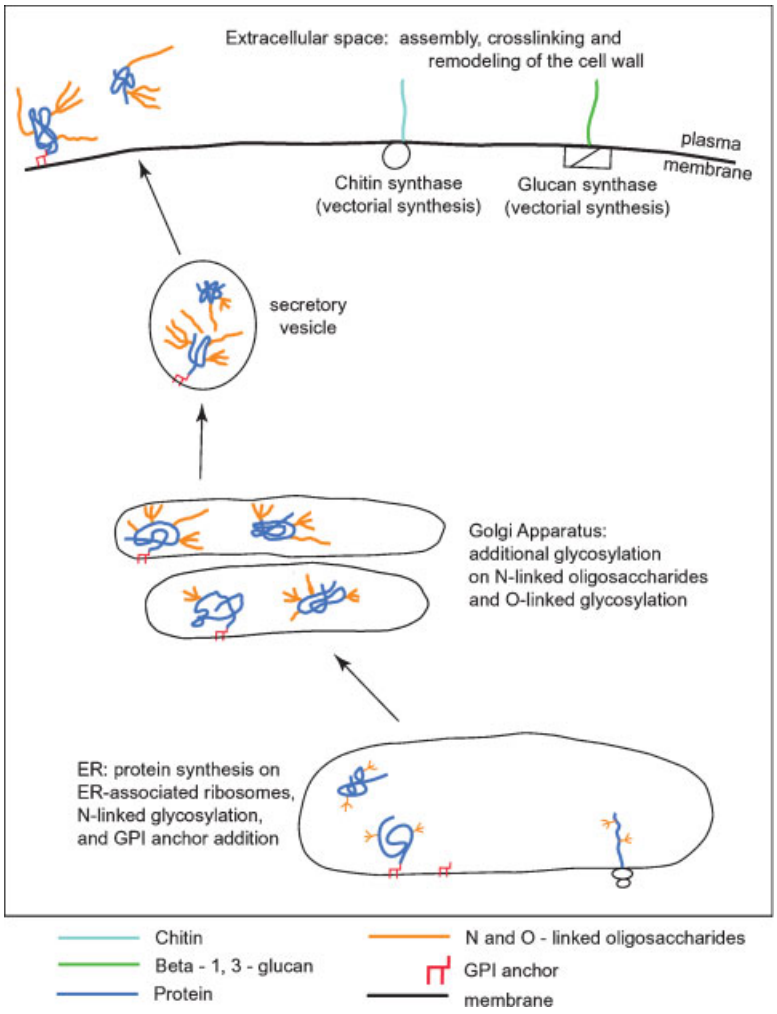

Figure 2. Cell wall biosynthesis. The glucan and chitin cell wall components are synthesized on the plasma membrane and extruded into the cell wall space during their synthesis. Glycoprotein synthesis begins in the endoplasmic reticulum with the cotranslational addition of N-linked oligosaccharides. GPI anchors are added to GPI-anchored proteins in the endoplasmic reticulum. In the Golgi apparatus, the glycosyltransferases further modify the proteins by the addition of sugars to generate O-linked oligosaccharides and to extend N-linked oligosaccharides. The glycoproteins are secreted into the cell wall space where they are integrated into the cell wall structure. The various components of the cell wall are cross-linked together in the cell wall space by cell-wall-associated glycosylhydrolases and glycosyltransferases.

enzyme may catalyze the majority of chitin synthesis under normal growth conditions.[22]

Because of the structural integrity that chitin provides the fungal cell, chitin synthesis has been considered an excellent target for anti-fungal agents. The best-known chitin synthesis inhibitors are the naturally occurring nikkomycins and polyoxins, as well as their synthetic derivatives. The nikkomycins and polyoxins are analogs of the chitin synthase substrate, UDP-*N*-acetylglucosamine, and function as competitive inhibitors for chitin synthase.[24] However, treatments with nikkomycins and polyoxins have not proved effective in controlling mycoses.[25] The nikkomycins and polyoxins are, however, often used in conjunction with other anti-fungal agents in treatment regimens. The ineffectiveness of nikkomycins and polyoxins is thought to be due to the limited uptake of the inhibitors into the cytoplasm of the fungal pathogen. Currently, fungicides that specifically target the chitin component of the cell wall have found limited use in therapeutic settings.

The glucan component of the fungal cell wall

Glucan is the major structural polysaccharide of the fungal cell wall, constituting approximately 50–60% of the wall by dry weight.[26,27] Polymers of glucan are composed of repeating glucose residues that are assembled into chains through a variety of chemical linkages. In general, between 65% and 90% of the cell wall glucan is found to be beta-1,3-glucan, but other glucans, such as beta-1,6-, mixed beta-1,3- and beta-1,4-, alpha-1,3-, and alpha-1,4-linked glucans, have been found in various fungal cell walls.[28–30] The beta-1,3-glucan serves as the main structural constituent to which other cell wall components are covalently attached. As a result, the synthesis of beta-1,3-glucan is required for proper cell wall formation and the normal development of fungi.

Initial studies examining the synthesis and composition of glucan were done in *S. cerevisiae* and *Candida albicans*. These studies demonstrated that yeast cell walls contain branched beta-1,3- and beta-1,6-glucans.[31] Recent studies

have shown that the cell walls of many filamentous fungi, including *N. crassa* and *A. fumigatus*, do not contain beta-1,6-glucan.[23,32]

Polymers of beta-1,3-glucan, like those of chitin, are generated by enzyme complexes associated with the plasma membrane and extruded into the extracellular space by means of vectorial synthesis.[33–35] As with chitin, this mode of synthesis promotes the association of nascent glucan chains within the cell wall space and facilitates their integration into the cell wall. This integration occurs at points of active cell wall synthesis, and the glucan synthase complexes, similar to those generating chitin, are primarily localized to areas of cell growth and budding or branching.[36–38] Glucan synthase catalyzes the formation of long linear chains of glucan, each composed of approximately 1,500 glucose residues connected via beta-1,3-linkages. Within each long glucan chain, the carbon-6 positions of approximately 40–50 glucose residues become sites at which additional beta-1,3-glucans are attached to generate a branched structure.[32,35] The particular enzymes responsible for creating the branched structure within the cell wall space have not been identified. The branched glucans can then be cross-linked together and to chitin and mannoproteins to provide the cell wall with mechanical strength and integrity.[39]

The genes encoding the components of the beta-1,3-glucan synthase machinery were first identified in *S. cerevisiae*, which contains two known catalytic subunits and one regulatory protein.[37,40,41] The *S. cerevisiae FKS1* and *FKS2* genes encode two functionally redundant catalytic subunits of the glucan synthase complex. Genetic analyses have demonstrated the involvement of each in glucan synthesis and cell wall formation. Disruption of either the *FKS1* or *FKS2* gene yields mutants with slow growth rates and cell wall defects.[40,41] The simultaneous deletion of *FKS1* and *FKS2* is lethal.[41] This finding is consistent with the catalytic subunits having overlapping functions and illustrates the importance of beta-1,3-glucan production for yeast survival. Disruptions of the *S. cerevisiae RHO1* gene, which encodes the Rho1 GTPase regulatory subunit, have demonstrated that it is essential for survival.

Using the work from the yeast system as a model, the glucan synthase components of many filamentous fungi are now being identified. The *FKS* and *RHO1* genes are highly conserved amongst fungi, and genome analyses have enabled the rapid identification of homologs in *A. fumigatus* and *N. crassa*. The *A. fumigatus* and *N. crassa* genomes each contain one catalytic subunit gene and one gene encoding a Rho1 GTPase regulatory subunit. The *A. fumigatus FKS1* and *RHO1* genes have been cloned and individually disrupted. Both genes are required for cell viability.[38]

The inhibition of beta-1,3-glucan synthesis has been extensively pursued as a means of disrupting wall formation and prevent fungal growth. A family of anti-fungal agents, known as the echinocandins, has been developed for clinical use. The echinocandins, which include caspofungin, micafungin and anidulafungin, are non-competitive inhibitors of the beta-1,3-glucan synthase complex.[42] Although the exact mechanism of inhibition is not fully understood, the echinocandins are known to bind to the glucan synthase catalytic subunit.[43] Treatment with the echinocandins results in cell swelling and lysis at areas of active cell wall synthesis and the echinocandins have emerged as a promising therapy for aspergillosis and candidiasis.[43,44]

The glycoprotein component of the fungal cell wall

All fungal cell walls have a protein component that is tightly interwoven within the chitin and glucan-based structural matrix. Proteins are reported to account for roughly 30–50% of the *S. cerevisiae* and *C. albicans* cell walls by dry weight.[26,45] The amounts of protein within the cell walls of filamentous fungi have been estimated to represent approximately 20–30% of the cell wall by mass. A recent empirical determination of the protein content of the *N. crassa* hyphal cell wall demonstrated the wall to be approximately 15% protein by dry weight.[46]

Most cell wall proteins are glycoproteins that have passed through the secretory pathway in transit to the cell wall. An interesting and somewhat perplexing phenomenon is the presence of ''intracellular'' proteins within the cell wall. Traditional cytoplasmic proteins, which were once considered to be contaminants in fungal cell wall preparations, are now gaining acceptance as true cell wall constituents. Proteomic studies with various fungi have identified a number of small cytosolic and mitochondrial proteins associated with the cell wall. Heat-shock proteins and glycolytic enzymes have been repeatedly detected and there is evidence indicating that they are cross-linked into the cell wall.[46–52] The functions of these non-traditional cell wall proteins are currently unknown.

Traditional cell wall proteins are glycoproteins, which are extensively modified with N-linked and O-linked oligosaccharides. The structures of the oligosaccharide chains attached to these glycoproteins differ amongst fungi. The cell walls of *S. cerevisiae* and *C. albicans* contain mannoproteins, which are glycosylated with chains rich in mannose, known as mannans.[26,45] In contrast, the glycoproteins of *N. crassa* and *A. fumigatus* contain galactomannan structures, composed of both mannose and galactose residues.[53–55] In addition to these modifications, some cell wall proteins receive a glycosylphosphatidylinositol (GPI) anchor. The GPI anchor, a lipid and oligosaccharide-containing structure, is added to select proteins that contain a C-terminal signal sequence and serves to direct and localize these proteins to plasma membrane and cell wall.

Most cell wall proteins are integrated into the wall via covalent linkages between the sugars present at the

N- and O-linked sites and/or in the GPI anchor with those in the polymers of chitin or glucan. Cell wall proteins function in maintaining cell shape, mediating adhesion for cell migration and fusion, protecting the cell against foreign substances, mediating the absorption of molecules, transmitting intracellular signals from external stimuli, and synthesizing and remodeling cell wall components.

The processes of N- and O-linked protein glycosylation are conserved among eukaryotes. Most glycoproteins possess a typical N-terminal signal peptide, which allows them to enter the secretory pathway. As they are synthesized, the proteins are extruded into the lumen of the endoplasmic reticulum (ER), where the processes of N- and O-linked glycosylation begin. During translocation of the protein into the lumen of the ER, a large, branched oligosaccharide structure is added to asparagine residues found in N-X-S and N-X-T sequence elements, where N is the acceptor asparagine, X represents any amino acid, and S and T are serine and threonine residues, respectively. The N-linked oligosaccharide contains N-acetylglucosamine (GlcNAc), mannose (Man), and glucose (Glc) residues, and is transferred to the protein en bloc from a dolichol lipid donor. The synthesis of this N-linked core oligosaccharide structure occurs on the ER membrane and involves the sequential addition of sugar residues to a dolichol lipid moiety. The addition of each sugar is mediated by a specific glycosyltransferase, which uses a nucleotide-sugar or dolichol-sugar intermediate as a substrate. The genes encoding several of these sugar transferases have been identified and are collectively referred to as asparagine-linked glycosylation (*ALG*) genes.[56] The attachment to the dolichol lipid group serves to tether the growing oligosaccharide chain to the membrane and provides the activation energy required for the glycosylation reaction. The completed core structure consists of a branched olgiosaccharide. The oligosaccharide is co-translationally transferred to the nascent protein by the N-oligosaccharyltransferase complex, which catalyzes the formation of a glycosidic bond between the first GlcNAc in the chain and the NH_2 group of the target asparagine.

Once attached to the protein, the N-linked core structure is subject to extensive modification in the ER and Golgi apparatus, which includes the trimming and addition of sugars to the core precursor. It is this additional processing that accounts for the diversity of the N-linked sugar components present on glycoproteins. In *S. cerevisiae* and *C. albicans*, mannoproteins are generated by the addition of long chains of alpha-1,6-linked mannoses with short branches of alpha-1,2 and alpha-1,3 mannoses.[31] In *N. crassa* and *A. fumigatus*, the N-linked structures are primarily modified with a galactomannan component.[53–55] This proposed N-linked galactomannan structure consists of an alpha-1,6-linked mannose core with alpha-1,2-linked mannose side chains, which are terminated with a variable number of beta-1,5-linked galactofuranose residues. The Golgi resident enzymes that process and elongate the N-linked oligosaccharides are encoded by members of the *KTR/KRE/MNT* and *MNN* gene families. In *S. cerevisiae*, the *KTR/KRE/MNT* and *MNN* families each consist of nine genes, many of which have been shown to participate in N-linked oligosaccharide synthesis.[57–65] In addition to their role in N-linked processing, many members of these gene families are also involved in the synthesis of the O-linked glycan structure.

The vast majority of information concerning the synthesis and addition of O-linked sugar structures in fungi comes from the work done in *S. cerevisiae*. In *S. cerevisiae*, O-linked glycan synthesis begins in the ER with the direct addition of a single mannose to select serine and/or threonine residues in the protein. This initial reaction is catalyzed by a family of protein O-mannosyltransferases (PMTs), which use dolichol-phosphate-mannose as a substrate. The *S. cerevisiae* genome is predicted to encode seven potential PMTs, five of which have been shown to be involved in O-linked glycosylation.[66,67] The remainder of oligosaccharide synthesis and elongation occurs in the Golgi apparatus and are mediated by the *KTR/KRE/MNT* and *MNN* families of mannosyltransferases. In *N. crassa*, and presumably *A. fumigatus*, the O-linked structure is slightly different in that it contains a galactose component. These O-linked structures are neither branched nor as long as those of their N-linked glycan counterparts.

Genetic analyses of *S. cerevisiae* have resulted in the isolation of several mutants defective in the processes of N- and O-linked glycosylation. These studies have shown that many of the gene products in these pathways have overlapping or redundant functions, consistent with the presence of multiple, large gene families involved in glycan synthesis, transfer and modification. In most instances, the disruption of a single gene involved in any one step may result in a modest reduction in the efficiency of glycosylation, but does not completely abolish the process or result in the death of the cell. However, the simultaneous disruption of multiple components of the pathways yields more severe phenotypes, which can include osmotic sensitivity, defects in cell wall composition and integrity, and cell death. These results are not unexpected given the importance of glycosylation for the proper conformation, localization and function of cell wall proteins.

In addition to N- and O-linked oligosaccharide modifications, a number of cell wall glycoproteins receive a glycosylphosphatidylinositol (GPI) anchor. GPI-anchored proteins, like other traditional cell wall proteins, have an N-terminal signal peptide to direct them to the secretory pathway and several N- and O-linked glycosylation sites. Those proteins destined to receive a GPI anchor also have a distinct GPI anchor addition signal in their carboxyl terminus. The GPI anchor signal sequence is a four-part motif, which begins with an unstructured, hydrophilic region of approximately eleven amino acids, followed by a region of three or four small amino acid residues, termed the omega site, a spacer region of approximately six

moderately polar amino acids, and concludes with a hydrophobic region of about ten amino acids at the carboxyl terminus of the pro-protein.[68,69] This signal sequence is recognized by a protein complex located in the membrane of the ER, known as the GPI transamidase. The GPI transamidase cleaves the target protein at the omega site and mediates the transfer of the pre-assembled GPI anchor structure onto the newly generated C terminus of the protein. The GPI anchor serves to localize the protein to the plasma membrane and/or cell wall and, in many instances, is also required for protein stability and function.

The structure of the GPI anchor has been extensively studied in yeast, trypanosomes and cultured mammalian cells. The GPI anchors present in these systems, and presumably all eukaryotes, share a common core structure.[69–71] This basic structure consists of a phosphatidylinositol moiety with an attached oligosaccharide chain that is terminated with a phosphoethanolamine group. The phosphatidylinositol is attached to either a ceramide- or a diacylglycerol-based lipid element. The inositol has a glucosamine residue attached to it through an alpha-1,6-linkage, which in turn is linked to a chain of three mannose residues. These mannoses are attached through an alpha-1,4-linkage (first mannose to glucosamine), an alpha-1,6-linkage (between the first and second mannoses), and an alpha-1,2-linkage (between the second and third mannoses). A phosphoethanolamine present on the carbon-6 position of the third mannose serves as the point of attachment to the recipient protein. The diversity amongst the GPI anchors from different organisms is generated from the presence of various side chains or substituents on this core structure.

The process of GPI anchor addition is mediated by approximately twenty different proteins, which are organized into biosynthetic complexes in the ER membrane. Some proteins possess defined enzymatic activities, while others function as auxiliary factors or serve structural roles within a given complex.

Genetic analyses have demonstrated that all but one of the genes in the GPI anchor biosynthetic pathway are essential for viability in *S. cerevisiae*. Deletion of the *GPI7* gene, the only *S. cerevisiae* GPI anchor biosynthetic gene that isn't required for viability, yields mutants with pronounced morphological defects, including alterations in cell wall biosynthesis, remodeling and integrity.[72]

Several of the GPI anchor biosynthetic pathway genes have recently been cloned in *N. crassa*.[46,73] Null mutations within some of the *N. crassa* GPI anchor biosynthetic pathway genes produce viable mutants with altered hyphal and colony morphologies, restricted rates of growth, and pronounced cell wall defects, while null mutations in other *N. crassa* GPI anchor biosynthetic genes are lethal. Clearly, as in *S. cerevisiae*, GPI-anchored proteins are required for the biosynthesis and integrity of the cell wall of *N. crassa*.

Various computer-modeling programs have been developed that can predict potential GPI anchor addition sites in the primary sequences of candidate proteins. One such program, the fungal big-π predictor, has been optimized for use with fungal proteins.[74] In a large-scale study, the big-π predictor was used to analyze fungal proteomes and identified 59, 28, 169, 74 and 87 genes encoding GPI-anchored proteins in the genomes of *S. cerevisiae, Schizosaccharomyces pombe, C. albicans, Aspergillus nidulans* and *N. crassa*, respectively.[75] Some of these proteins have since been verified as being GPI-anchored in the *S. cerevisiae, A. fumigatus* and *N. crassa* systems.[46,76,77] Many of the proteins predicted to have GPI anchors putatively function as glycosylhydrolases, glycosyltransferases, and peptidases, which are likely to participate in cell wall synthesis and remodeling.[78–80] Based upon genetic analyses of mutants affected in GPI anchoring, the roles of known GPI-anchored proteins, and the putative functions of other proteins predicted to be GPI-anchored, it is clear that GPI-anchored proteins are critical for the formation and maintenance of the cell wall. Thus, disruptions in the GPI anchor pathway would be expected to have profound effects on the structure and function of the fungal cell wall.

Cell wall dynamics

The dynamic nature of the fungal cell wall originates from the concerted actions of numerous glycoproteins present within the structure. The individual functions of most cell wall-associated proteins have not been conclusively determined. However, based upon computer modeling and homology-based analyses, the proteins can be grouped into two broad categories: wall-associated enzymes (WAEs) and "structural" proteins. The WAEs are proteins with apparent catalytic activities. Many of these enzymes are actively involved in cell wall synthesis and remodeling.[81] The WAEs include chitinases, glucanases and peptidases, which hydrolyze and breakdown cell wall components, as well as glycosyltransferases which are involved in the synthesis and cross-linking of wall polymers. The enzymatic activities of these proteins must be appropriately balanced to provide the cell wall with adequate elasticity to allow new growth, budding or branching, and sufficient strength to guard against cell lysis.

The "structural" proteins become incorporated into the cell wall, but lack enzymatic activity. In general, these "structural" proteins are relatively small in size and often lack clearly defined functional domains. Many of these proteins may play roles in cell migration, adhesion, fusion and mating, or serve as scaffolding proteins to which other cell wall components are attached.[76]

Several WAEs have been identified in *S. cerevisiae* and *A. fumigatus* and shown to be involved in cell wall remodeling.

These proteins, which break (hydrolases) or form (transferases) bonds within and between cell wall polysaccharides, are the primary contributors to wall dynamics. A number of glucanases and chitinases have been purified from the cell walls of *S. cerevisiae* and *A. fumigatus* and demonstrated to have either endo- or exo-glycolytic activity.[82–87] The genomes of yeast and filamentous fungi contain a number of genes encoding chitinases and glucanases. These hydrolases are likely to have overlapping functions but, in some cases, they can be shown to play a role in cell wall dynamics. For example, the *S. cerevisiae CTS1* and *CTS2* genes encode endochitinases that function in budding/cell separation and spore wall assembly, respectively.[86,87]

The glycosyltransferases present in the *S. cerevisiae* and *A. fumigatus* cell walls have both glycolytic and synthetic activities, enabling them to cleave molecules and subsequently rejoin or ''transfer'' them into larger polymers. The transferases encoded by the *S. cerevisiae GAS* and *A. fumigatus GEL* gene families are among the best characterized. These enzymes have been shown to internally cleave a molecule of beta-1,3-glucan and transfer the newly generated reducing end to the non-reducing end of another beta-1,3-glucan molecule.[78–80] This transferase reaction establishes a new beta-1,3-linkage and can result in the elongation of beta-1,3-glucan chains.

In *S. cerevisiae*, both the WAEs and ''structural'' proteins have been classified based upon the type(s) of bonds that mediate their association with the cell wall. Some cell-wall-associated proteins are non-covalently attached to the cell wall and can be extracted with salt, alkali and ionic detergents. These proteins are referred to as soluble cell wall (SCW) proteins. Other cell wall proteins are covalently linked to the wall matrix, presumably through the numerous N- and O-linked glycans present on these proteins and resident polymers of chitin and glucan. Such integral proteins are known as covalently linked cell wall (CCW) proteins. The purification of these CCW proteins requires the prior enzymatic or chemical digestion of the cell wall. Because many *S. cerevisiae* cell wall protein have unknown functions, they have been named according to the SCW or CCW designations.

Demonstrating the roles of individual WAEs or ''structural'' proteins in cell wall remodeling and integrity is confounded by an apparent redundancy that exists among many cell-wall associated proteins. The disruption of a gene encoding a cell wall protein rarely results in an obvious mutant phenotype. Cell wall defects are more easily observed following the deletion of multiple WAE or ''structural'' protein-encoding genes.

In yeast and *Aspergillus*, a clear picture of the cell wall is emerging as a site of active construction and remodeling. The collaborative actions of cell wall proteins maintain a continual balance within the dynamic cell wall, as components are formed by synthases, modified by WAEs, and arranged and stabilized by a series of ''structural'' proteins.

The current status of clinical control of systemic mycoses

This review has focused on the biosynthesis and organization of the fungal cell wall, and anti-fungal agents that target cell wall biosynthesis have been described when discussing different aspects of the wall. Most therapeutics currently in use for the treatment of mycoses target other aspects of fungal biology.[24,25,43,44] The most-commonly used classes of anti-fungal agents, the azoles and polyene antibiotics, target the presence and biosynthesis of ergosterol, the fungal plasma membrane sterol that plays the same role in the fungal membrane that cholesterol plays in the human plasma membrane. The polyene antibiotics, amphotericin B and nystatin, selectively bind to ergosterol and disrupt the fungal plasma membrane. The azole anti-fungal agents, fluconazole, ketoconazole, itraconazole, posaconazole, voriconazole and ravuconazole, affect the biosynthesis of ergosterol. The azole anti-fungal agents lead to an altered plasma membrane composition and affect membrane functions. Griseofulvin, which has been used as an anti-fungal agent for many years, is thought to affect the microtubule assembly process and thereby disrupt fungal cell division. The sordarins are a class of anti-fungal agents that has received some recent interest. They inhibit protein synthesis by interacting with translation elongation factor 2. Of the generally used anti-fungal agents, only the echinocandins target the fungal cell wall.

Future directions

The fungal cell wall biosynthetic process has a number of steps that are well-suited as targets for anti-fungal agents. The structure of the cell wall is unique to the fungi. The biosynthetic enzymes that carry out the steps involved in chitin and glucan synthesis, and the glycosyltransferases that cross-link these polymers in the cell wall, lack homologs in the human genome. The only step that is currently targeted by commercially available anti-fungal agents is the beta-1,3-glucan synthase complex, which is inhibited by the echinocandins. The chitin synthase complex is a second target that might well be exploited as a target for anti-fungal agents. The nikkomycins and polyoxins have found some usage in conjunction with other anti-fungal agents, but the development of agents that affect chitin synthase as noncompetitive inhibitors, much like the echinocandins affect glucan synthase, would be a welcomed addition to the anti-fungal arsenal. The various mannosyltransferases and other glycosyltransferases used in the Golgi apparatus to synthesize the N-linked and O-linked oligosaccharides and mannans that are attached to the cell wall glycoproteins represent a third target for the development of anti-fungal drugs. Fungal mutants that are affected in the biosynthesis of these oligosaccharides are severely affected in their growth and pathogenicity.[60,62,66,67,88,89] The fourth target that could be exploited for the development of anti-fungal drugs is the process of generating and attaching a GPI

anchor to cell wall proteins. Mutations affecting GPI anchor biosynthesis and attachment are lethal, demonstrating the importance of the GPI-anchoring process in cell wall biosynthesis. Although the GPI anchor core structure is conserved between humans and fungi, there are differences in the number and placement of additional sugar residues onto the core structure.[69] For example, the addition of a fourth mannose to the core structure is a required step for GPI anchor synthesis in yeast cells but not in human cells. Drugs that specifically target such fungal-specific steps in GPI anchor biosynthesis would be expected to be effective in controlling fungal growth. Yet a fifth target for anti-fungal drugs might be the structure of the wall itself. The anti-fungal agent pradimicin, which binds to the terminal mannose of fungal cell wall oligosaccharides in a calcium-dependent manner and disrupts the plasma membrane, is an example of such an antibiotic. Pradimicin has not been used for treatment of systemic mycoses because it can lead to liver damage. Derivatives of pradimicin, or other drugs that specifically bind to fungal cell wall structures, might be good candidates for anti-fungal agents. The sixth, and perhaps best, cell wall target for an anti-fungal drug would be the various cell wall glycosyltransferases that function in the extracellular space to assemble and cross-link the cell wall components together. Agents that inhibit the activities of the transferases that cross-link the beta-1,3-glucans with each other and with the chitin and cell wall glycoproteins would be particularly good candidates for the development of anti-fungal drugs. Such drugs would not have to cross through the plasma membrane to get to their site of action and would target a critical step in cell wall biosynthesis. Penicillin and its analogs function in a very similar way to inhibit the cross-linking of bacterial cell wall components.

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