MINIREVIEW

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Biofilm Exopolysaccharides of Pathogenic Fungi: Lessons from Bacteria*

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Exopolysaccharides play an important structural and functional role in the development and maintenance of microbial biofilms. Although the majority of research to date has focused on the exopolysaccharide systems of biofilm-forming bacteria, recent studies have demonstrated that medically relevant fungi such as *Candida albicans* and *Aspergillus fumigatus* also form biofilms during infection. These fungal biofilms share many similarities with those of bacteria, including the presence of secreted exopolysaccharides as core components of the extracellular matrix. This review will highlight our current understanding of fungal biofilm exopolysaccharides, as well as the parallels that can be drawn with those of their bacterial counterparts.

Biofilms are communities of microorganisms growing within a self-produced extracellular matrix composed of proteins, extracellular DNA, lipids, and mono- and polysaccharides. Seminal studies in bacterial systems, as detailed in the other articles within this minireview compendium, have identified a critical role for exopolysaccharides in the establishment, structure, and function of biofilms. Emerging evidence suggests that biofilm formation is not restricted to bacteria and that pathogenic fungi, such as *Aspergillus fumigatus* and *Candida albicans*, also produce biofilms during colonization and infection. Fungal biofilms mediate adherence to host tissues and biomedical devices, and provide protection from host immune defenses and antifungal therapy.

C. albicans, the most common fungal pathogen of humans, forms extensive biofilms on medical devices and mucosal surfaces during infection (1, 2). Studies of the biofilm matrix have revealed a cooperative role of β-1,3-glucans with a mannan-glucan complex in the development and maintenance of biofilm structure (3, 4). Although similar polysaccharides are also found within the fungal cell wall, the synthesis of the matrix polysaccharides is governed by pathways independent of those that mediate cell wall synthesis. β-1,3-Glucans within the *C. albicans* biofilm matrix play an important role in sequestration of antifungal agents (5) and in masking fungal cells from recognition by neutrophils (6). In contrast, biofilms formed by the mold *A. fumigatus* have been reported during pulmonary infection (7) and are dependent on the exopolysaccharide galactosaminogalactan (GAG)[3] (8). GAG is a partially deacetylated heteropolymer of α-1,4-linked galactose and *N*-acetyl galactosamine, which mediates adhesion between both fungi and also to other surfaces as well as protection against host immune defenses (8–11). GAG is synthesized by the protein products of a conserved cluster of five genes that is present in a number of plant and human fungal pathogens (11). Despite a lack of sequence homology with bacterial exopolysaccharide biosynthetic enzymes, there are striking similarities in the function and organization of these fungal exopolysaccharide biosynthetic proteins and the role of the resulting glycans in biofilm formation, drug resistance, and immune evasion. In this review, we will summarize our current understanding of the composition, biosynthesis, and function of fungal biofilm exopolysaccharides produced by the pathogenic fungi, *A. fumigatus* and *C. albicans*, and compare and contrast these mechanisms with those of common pathogenic bacteria.

A. fumigatus Biofilms

Biofilm formation by the filamentous fungus *A. fumigatus* has been recently described during growth *in vitro* and *in vivo* (7, 12). As with bacteria, the filamentous hyphae of *A. fumigatus* grow embedded within an extracellular polymeric substance (7, 12). This extracellular matrix mediates adherence to inorganic substrates and host cells and enhances resistance to host defenses and antifungal agents. This matrix is a heterogeneous substance composed of extracellular DNA, proteins, lipids and polyols, and exopolysaccharides including α-glucans, galactomannan and the glycan GAG (7, 13).

A. fumigatus Biofilm Exopolysaccharides

Of the three exopolysaccharides found within *A. fumigatus* biofilms, the role of GAG has been studied in the greatest detail. GAG is an α-1,4-linked heteropolysaccharide composed of galactose and partially deacetylated GalNAc that is absent in *A. fumigatus* spores but is produced in abundance by growing hyphae (8, 11, 14). GAG plays a critical role in the maintenance of the extracellular matrix of *A. fumigatus* biofilms (8). Strains deficient in GAG production fail to produce extracellular matrix and are unable to form adherent biofilms on plastic or host cells *in vitro* (8, 9, 15). As with other biofilm exopolysaccharides, GAG plays an important role in evading host

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[3] The abbreviations used are: GAG, galactosaminogalactan; Gal, galactopyranose; PNAG, poly-β1,6-*N*-acetylglucosamine; EPS, exopolysaccharide; ROS, reactive oxygen species; AlgL, alginate lyase; ALS, agglutinin-like sequence.

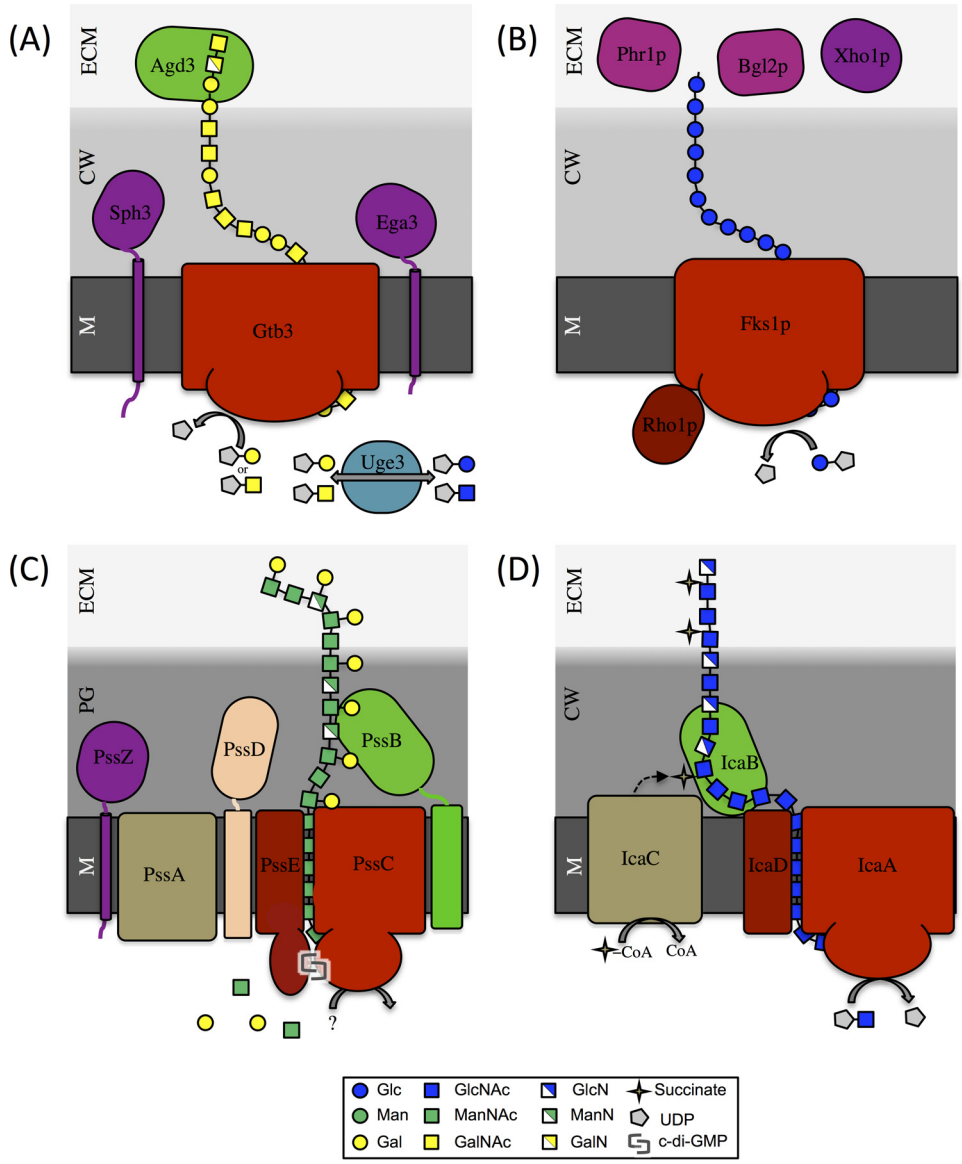

FIGURE 1. **Schematic representation of the GAG, β-1,3-glucan, EPS, and PNAG polysaccharide synthase from *A. fumigatus*, *C. albicans*, *L. monocytogenes*, and *S. epidermidis*, respectively.** In each panel, the glycosyltransferases and associated regulatory proteins are depicted in *red* and *dark red*, respectively; the deacetylase is in *light green*, the epimerase is in *light blue*, hydrolases are in *purple*, and other modifying enzymes are in *beige*. *M*, membrane; *CW*, cell wall; *ECM*, extracellular matrix; *Glc*, glucose; *Gal*, galactopyranose; *Man*, mannose; *c-di-GMP*, cyclic di-GMP.

defenses. As a result, strains deficient in GAG display attenuated virulence in mouse and invertebrate models of invasive aspergillosis (8, 9).

A functional role for the other polysaccharides found within the extracellular matrix of *A. fumigatus* biofilms has yet to be established. Galactomannan, which consists of a mannan core decorated with β-1,5-linked galactofuranose, is dispensable for biofilm formation, and galactofuranose-deficient mutants exhibit increased biofilm formation as a consequence of increased production of GAG (8, 10, 16). α-Glucans are abundant within the biofilm matrix; however, the role of this polysaccharide in biofilm formation has not been studied (13, 17–19).

Galactosaminogalactan Biosynthesis

Comparative transcriptomic studies of regulatory mutants deficient in GAG production identified a cluster of five co-regulated genes with predicted functions in polysaccharide synthesis and metabolism. Subsequent studies have confirmed a role for the protein products of many of these genes in the synthesis or processing of GAG. The presence of gene clusters resembling bacterial operons is relatively unusual in filamentous fungi, and often suggests that acquisition of these genes occurred through a horizontal gene transfer event. Bioinformatics analyses of available fungal genomes support this hypothesis, as the presence of a syntenic GAG gene cluster is found throughout a variety of taxonomically diverse fungal genomes (11).

Through bioinformatics, gene deletion experiments, and the study of recombinant enzymes, a model for GAG biosynthesis is emerging (Fig. 1). This model shares some similarity with those described for the synthase-dependent production of hexosamine-containing polysaccharides found within bacterial

TABLE 1
Select microbial exopolysaccharides that contain hexosamine components

Organism	Polymer	Operon	Structure
Aspergillus fumigatus	GAG	NA[a]	Partially deacetylated α-1,4-linked heteroglycan of Gal and GalNAc
Pseudomonas aeruginosa	Pel	*pel*	Partially deacetylated 1,4-heteroglycan of GlcNAc and GalNAc
Listeria monocytogenes	EPS	*pss*	β-1,4-Linked *N*-acetylmannosamine chain decorated with terminal α-1,6-linked Gal; possibly partially deacetylated
Streptococcus thermophilus	EPS	*eps*	Tetrasaccharide repeat with 1,3-linked β-Gal, β-Glc, and α-GalNAc with Gal α-1,6 linked to the central Glc
Staphylococcus epidermidis[b]	PNAG	*ica*	Partially deacetylated β-1,6-linked GlcNAc
Escherichia coli[b]	PNAG	*pga*	Partially deacetylated β-1,6-linked GlcNAc

[a] NA, not applicable.
[b] These species are included as Gram-positive and Gram-negative examples of the range of microorganisms that produce PNAG.

biofilms (Table 1). As *A. fumigatus* contains only a single plasma membrane, the model of GAG biosynthesis is most similar to exopolysaccharide synthesis by Gram-positive bacteria such as *Staphylococcus epidermidis* (20) and *Listeria monocytogenes* (Fig. 1) (21).

Amino Sugar Synthesis

Synthesis of GAG begins with the production of UDP-galactopyranose (Gal) and UDP-GalNAc from UDP-glucose and UDP-GlcNAc, respectively, by the bifunctional cytosolic epimerase Uge3 (8, 10). The activity and substrate specificity of this enzyme has been confirmed *in vitro* (10) and Uge3-deficient mutants produce no detectable GAG (8). The presence of an epimerase-encoding gene within the GAG biosynthetic gene cluster is unique to *A. fumigatus*. Although other bacteria such as *Pseudomonas aeruginosa* and *Streptococcus thermophilus* produce GalNAc-containing exopolysaccharides, the operons encoding the biosynthetic genes for these bacterial glycans do not include genes predicted to encode glucose epimerases (22, 23). In these bacteria, UDP-GalNAc is derived from other cellular metabolic pathways that are not specific to exopolysaccharide synthesis. This difference is best illustrated by experiments in which the entire *S. thermophilus eps* operon was expressed in *Lactococcus lactis*, which lacks endogenous GalNAc production (24). Although the operon was functional in *L. lactis*, the resulting polymer was altered in composition and was deficient in GalNAc, suggesting that this sugar is derived from other cellular processes and is not produced by enzymes encoded within the exopolysaccharide biosynthetic operon (24).

Glycan Chain Synthesis

Following synthesis by Uge3, UDP-Gal and UDP-GalNAc are predicted to be polymerized and exported from the cell through the action of a large transmembrane glycosyl transferase, Gtb3. Consistent with this model, disruption of *gtb3* results in a complete absence of GAG production, although this protein has not yet been purified and studied *in vitro*. The ability of a single glycosyl transferase to display specificities for multiple donor and recipient sugars has also been reported in studies of the *S. thermophilus* EPS system (24). As described above, transfer of the *eps* operon to a GalNAc-deficient bacteria results in the substitution of galactose for GalNAc within the mature polymer (24), suggesting that either Gal or GalNAc can serve as a suitable substrate during polymer synthesis. This promiscuity of transferase activity suggests that the acetyl group of GalNAc is not part of the recognition signal for the binding of nucleotide sugars by these enzymes. Bioinformatics analysis suggests that the transmembrane and glycosyl transferase regions of Gtb3 comprise less than half of the protein, with the transferase region predicted to be a member of the GT4 family of proteins as defined by the Carbohydrate-Active Enzymes database (25). It is possible that the domains encoded by these uncharacterized regions may participate in post-translational regulation of GAG synthesis. This model would be similar to the bifunctional BcsA and WssB enzymes that are responsible for the synthesis of cellulose and acetylated cellulose, respectively (26–29). In addition to the transferase domain, these proteins also contain a cyclic di-GMP receptor domain. Binding of the nucleotide is required for cellulose synthesis. In contrast, other bacterial systems have a separate regulatory protein that is associated with a transmembrane synthase. Examples of these regulatory proteins include IcaD/PgaD in the poly-β1,6-*N*-acetylglucosamine (PNAG) systems (20, 30) and PelD in the *P. aeruginosa* Pel system (31). Further studies are required to elucidate the function of the other regions of Gtb3.

Post-synthetic Polymer Modification

The newly synthesized GAG polymer, consisting of α-1,4-linked Gal and GalNAc, is deacetylated by the secreted, cell wall-associated enzyme Agd3 (11). Deacetylation results in the production of a polycationic glycan that associates with negatively charged residues on the cell wall of *A. fumigatus*, as well as mediates adherence to other anionic surfaces including host cells and plastics (11). Agd3-deficient mutants of *A. fumigatus* do not form biofilms, and produce fully acetylated GAG, which fails to adhere to the surface of the organism and is shed into the culture supernatant (11). Deacetylation occurs in the extracellular space as co-culture of an Agd3-deficient mutant with culture supernatants from the GAG-deficient Δ*uge3* mutant restores the production of cell wall-associated GAG and biofilm formation (11). Deacetylation of *N*-acetyl hexosamine polymers by enzymes belonging to the carbohydrate esterase 4 superfamily, such as Agd3, is also common in bacterial systems. In *S. epidermidis*, IcaB mediates the deacetylation of GlcNAc within the PNAG polymer following synthesis of the nascent glycan (32). As with *A. fumigatus* GAG, deacetylation is required for the formation of adhesive PNAG, and fully acetylated PNAG fails to adhere to the cell wall of *S. epidermidis* and cannot support biofilm formation (32). Interestingly, deletion of the genes involved in de-*N*-acetylation of PNAG in Gram-negative species results in different phenotypes. Deletion of *pgaB* in *Escherichia coli* results in loss of polymer export (33), whereas deletion of *bpsB* in *Bordetella bronchiseptica* results in a weaker biofilm with significant loss of the complex biofilm architecture (34). The Gram-negative organism *P. aeruginosa*

produces a heteropolymer of GlcNAc and GalNAc that is partially deacetylated (35). The *pel* operon contains a gene *pelA* that contains a carbohydrate deacetylase domain (36). Both deletion of *pelA* and mutation of catalytic residues that compromise deacetylase activity are associated with a loss of Pel-mediated biofilm production and an absence of detectable biofilm-associated Pel (36). In *L. monocytogenes*, the protein product of *pssB* has been hypothesized to mediate deacetylation of *N*-acetylmannosamine residues within the EPS of this Gram-positive organism (21). As with *pelA*, deletion of *pssB* resulted in a loss of cell aggregation and EPS production, suggesting that this putative deacetylase is also required for the production of functional polysaccharide in *L. monocytogenes* (21).

In addition to rendering GAG adhesive, deacetylation of the polymer plays an important role in other functions of this glycan, including virulence. Binding of GAG to the outer cell wall of *A. fumigatus* hyphae conceals β-1,3-glucan from recognition by the innate immune receptor Dectin-1 on dendritic cells, leading to reduced pro-inflammatory cytokine production by these cells (8). Cell wall-associated GAG also mediates resistance to killing by host neutrophil extracellular traps, likely through charge-mediated repulsion of cationic peptides embedded within the DNA matrix of these structures (9). Similar observations have been made with deacetylase-deficient mutants of *S. epidermidis*, in which loss of cell wall-associated PNAG results in enhanced susceptibility to neutrophil phagocytosis and to the cationic antimicrobial peptide LL-37 (32). Interestingly, a recent study suggests that the *P. aeruginosa* cationic exopolysaccharide Pel binds extracellular DNA within biofilms through charge-charge interactions (35). Whether GAG or other bacterial cationic polysaccharides play similar structural roles in their respective biofilms remains to be determined.

Glycoside Hydrolases

The GAG biosynthetic gene cluster contains two additional genes: *sph3* and *ega3*. Although Ega3 is predicted to encode a membrane-anchored glycoside hydrolase, it has yet to be functionally characterized *in vitro* or *in vivo*. Recent work has found that Sph3 is a member of a novel glycoside hydrolase family, GH135 (15). Recombinant Sph3 cleaves purified and cell wall-associated GAG *in vitro* and displays no activity against chitosan, another hexosamine-containing polymer (15). Sph3 is required for GAG production (15), although the mechanism by which this enzyme participates in GAG synthesis remains unknown. This seeming contradiction in which hydrolases or other degrading enzymes mediate polymer production has also been reported in several bacterial exopolysaccharide systems. In *L. monocytogenes*, *pssZ* is predicted to encode a membrane-bound hydrolase that is required for exopolysaccharide production by this organism (21). Similarly, in several cellulose-producing organisms, the production of cellulose endoglucanase (37, 38) or carboxymethylcellulase (39) enhances the production of cellulose, suggesting that these cellulose-degrading enzymes play an important role in glycan maturation and/or export through the cell wall. Finally, in *P. aeruginosa*, alginate lyase (AlgL) is thought to form part of the transport complex of proteins that serve as a conduit to move the polysaccharide alginate through the periplasm, and also degrades any misdirected polymer within this space (40). Deletion of *algL* results in disruption of the conduit complex and retention of alginate within the periplasm, as well as the absence of AlgL-mediated polymer degradation, leading to cell lysis and death (40).

Regulation of *A. fumigatus* Biofilm Formation

Relatively little is known about the regulation of biofilm formation and exopolysaccharide synthesis by *A. fumigatus*. External stimuli that induce or suppress biofilm formation have yet to be identified. Production of GAG is dependent on the developmental regulatory proteins MedA (8, 41, 42), StuA (8), and SomA (43). Activation of the cell wall integrity pathway through deletion of the phosphatases SitA (44) or PtcB (45) has been linked to suppression of biofilm formation and extracellular matrix production. Although in bacteria, there is a well established role for cyclic di-GMP as a signaling molecule that stimulates exopolysaccharide production, there is no evidence that fungi, including *A. fumigatus*, produce this molecule.

Candida albicans Biofilms

The formation of biofilms by the yeast *C. albicans* plays a key role in the pathogenesis of both mucosal and catheter-related bloodstream infections in humans (46). Biofilm formation by *C. albicans* has been well studied *in vitro* and in animal models and is similar to the process that has been described for pathogenic bacteria (47). First, planktonic yeast cells adhere to an appropriate substrate such as an epithelial cell surface or an intravascular catheter. Adhesion and cell-cell aggregation are mediated by an array of glycosylphosphatidylinositol-linked cell wall glycoproteins including Eap1p (48), Hwp1p (49), and members of the agglutinin-like sequence (Als) family of proteins, particularly Als1p and Als3p (50–52). This initial adhesion and aggregation of yeast cells is further stabilized by the formation of amyloid structures between Als proteins (53–55). Following adhesion, *C. albicans* biofilms undergo maturation through reproduction of yeast cells and morphologic switching of yeast to produce filamentous hyphal and pseudohyphal structures. In parallel, these organisms elaborate an extracellular matrix consisting of DNA, proteins, lipids and mono- and polysaccharides. Mature biofilms may undergo a dispersal phase in which organisms shift morphology back to the yeast form, and are then released to disseminate through the environment.

The Exopolysaccharides of *C. albicans* Biofilm Matrix

A recent analysis of the composition of *C. albicans* biofilm matrix has revealed that three polysaccharides play a complex and cooperative function in the assembly and maintenance of the *C. albicans* biofilm matrix. Surprisingly, β-1,3-glucan, the predominant polysaccharide found within the *C. albicans* cell wall, and the glycan best linked with biofilm-mediated drug resistance, is only a minor component of the biofilm matrix (4). The most abundant polysaccharides within the extracellular matrix are an α-1,6-linked mannan with α-1,2-linked side chains (85%) and linear β-1,6-glucans (14%). These two polysac-

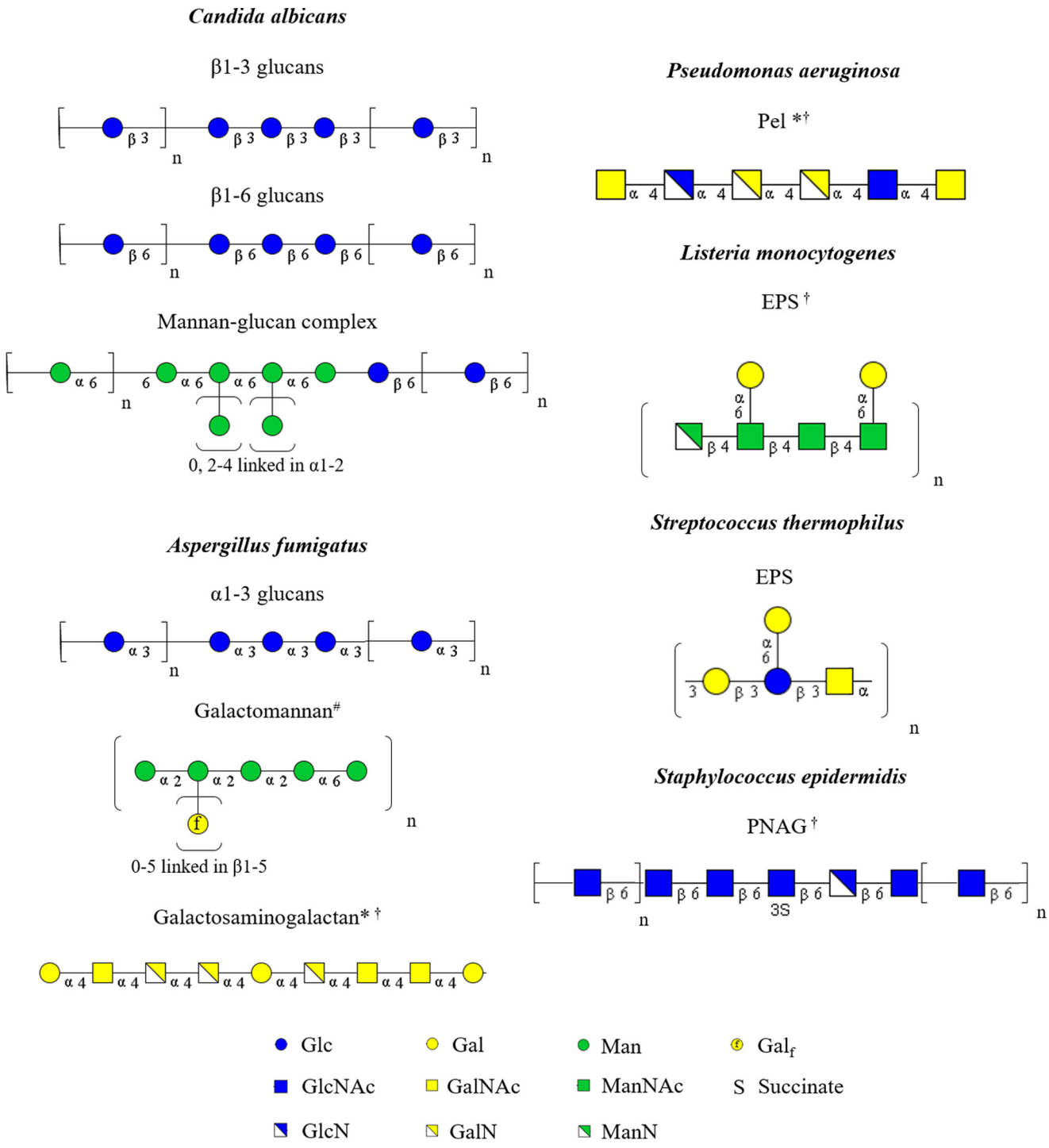

FIGURE 2. **Matrix exopolysaccharides discussed in this review.** Shown is a graphical representation of exopolysaccharides with *parentheses* indicating repeating elements. *Subscripts* indicate possible number of residues in each element. *Glc*, glucose; *Gal*, galactopyranose; *Man*, mannose; Gal_f, galactofuranose; *GalNAc*, *N*-acetyl galactosamine; *GalN*, galactosamine. *, no specific arrangement of residues or polymer length has been defined. #, galactofuranose side chains of galactomannan can be linked to any of the indicated mannan residues. †, the degree of deacetylation of these polymers is not known.

charides were co-isolated, suggesting that they form a mannan-glucan complex (4). The components of the mannan-glucan complex exhibit structural features that are unique to the extracellular matrix and are not found within their cell wall counterparts. Mannans within the extracellular matrix are much longer (up to 12,000 residues) as compared with those within the cell wall (~150) (4). Similarly, matrix β-1,6-glucans were found to be linear, in contrast with the highly branched structures found within the cell wall (4). Collectively, these observations suggest that in *C. albicans*, as has been established in bacteria (56, 57), the assembly of the cell wall and extracellular matrix are distinct processes. See Fig. 2 for graphical representations of matrix exopolysaccharides discussed in this review.

β-1,3-Glucan Biosynthesis

The synthesis of β-1,3-glucan is the best studied of the *C. albicans* matrix polysaccharides. Production of β-1,3-glucan for both cell wall and matrix production in *C. albicans* is mediated by a protein complex composed of the transmembrane glucan synthase Fks1p (58–60) and the regulatory G-protein Rho1p (61, 62) (Fig. 1). Fks1p is predicted to be a member of the GT48 superfamily. The presence of a regulatory protein associated with a transmembrane synthase differs from the *A. fumigatus* GAG pathway but is common in bacterial exopolysaccharide pathways as described above. Activation of the *C. albicans* Fks1p-Rho1p complex leads to the production of β-1,3-glucan by Fks1p from intracellular UDP-glucose through the addition of glucose residues to the non-reducing end of the glycan (63, 64). The emerging polymer is then thought to be transported to the extracellular space via a channel formed by the transmembrane domains of Fks1p.

Delivery of β-1,3-glucan to the extracellular matrix is then governed, at least in part, by a novel glucan-modifying pathway composed of at least three proteins: two glycosyltransferases, Bgl2p and Phr1p, and a glucanase, Xog1p (65). Deletion of any of the genes encoding these proteins results in a 10-fold or more decrease in matrix β-1,3-glucan content and the formation of biofilms that are more easily disrupted (65). Consistent with the decrease in matrix β-1,3-glucan, biofilms formed by these mutants are less able to sequester fluconazole and exhibit increased sensitivity to this agent (65). Despite these dramatic changes in matrix composition, these mutants produce normal levels of β-1,3-glucan within their cell wall, suggesting that the activity of these enzymes was specific for matrix-associated β-1,3-glucan (65). Overexpression and double-deletion studies suggest that these enzymes play complementary roles in glucan delivery (65). The exact mechanism by which these three enzymes mediate delivery of β-1,3-glucan to the extracellular matrix remains to be determined; however, they may participate in the release and modification of cell wall glucans for transport and deposition within the extracellular matrix.

β-1,3-Glucan plays an important role in biofilm-mediated protection of *C. albicans* from immune defenses and antifungals. The production of β-1,3-glucan by *C. albicans* biofilms inhibits the production of reactive oxygen species (ROS) by neutrophils and protects fungal cells from neutrophil killing (6). Treatment with glucanase enhances neutrophil ROS production and renders biofilms more susceptible to killing, whereas the addition of a soluble β-glucan to early biofilms reduces ROS production and protects cells from neutrophil killing, suggesting a role for β-glucans in mediating protection against neutrophils (6). A similar role in suppressing oxidative burst and neutrophil killing has been reported for the *P. aeruginosa* exopolysaccharide PslG, through reducing complement binding and opsonization of bacteria within biofilms (66). β-1,3-Glucan directly enhances antifungal resistance by the binding and sequestration of fluconazole, preventing its intracellular penetration and antifungal activity (5, 67, 68). Sequestration of antimicrobials by bacterial exopolysaccharides has also been reported in *P. aeruginosa*. In this organism, binding of tobramycin by alginate has also been demonstrated; however, the significance of tobramycin sequestration in mediating tobramycin resistance is the subject of some debate (69).

Biosynthesis of the Mannan-Glucan Complex

The synthetic pathways governing production of the mannan-glucan complex are largely unknown. Targeted gene deletion studies have identified a number of candidate genes whose protein products are required for the synthesis of this glycan complex, although the function of these proteins has not been studied to date. Deletion of *vig1* and *kre5* genes was found to be required for β-1,6-glucan synthesis, whereas loss of *alg11*, *mnn9*, *mnn11*, *van1*, *mnn4-4*, *pmr1*, and *vrg4* resulted in a reduction in the production of matrix α-mannan (3). Although the function of the protein products of these genes requires further investigation, this study provided insight into the contributions of the three major matrix polysaccharides to *C. albicans* biofilm formation. Loss of any one of the three polysaccharides was associated with a marked reduction in the extracellular matrix, lower levels of the other two polysaccharides, and enhanced susceptibility to fluconazole (3). Co-purification experiments suggested that the three polysaccharides physically associate in the extracellular matrix (3). Assembly of this polysaccharide matrix likely occurs in the extracellular space, as biofilm matrix formation was restored in mixed cultures of complementary mutant strains (3). Collectively, these findings suggest a model where the mannan-glucan complex and β-1,3-glucan are assembled after export from the cell and suggest that they play a cooperative role in the production of biofilm matrix. This cooperative function of multiple exopolysaccharides in the biofilm matrix is analogous to the roles of Pel and Psl in biofilm formation by *P. aeruginosa* (70). It has been suggested that these glycans function in a complementary fashion to govern the strength and viscosity of biofilm matrix in this bacteria.

Regulation of *C. albicans* Biofilm Formation

The regulation of *C. albicans* biofilm formation has been studied in greater detail than *A. fumigatus*. In *C. albicans*, biofilm formation and matrix synthesis are regulated by at least six transcription factors that govern adhesion, morphologic switching, and extracellular matrix production. Notable among these is Bcr1p, which positively regulates the expression of numerous adhesins during the initial phase of biofilm formation (71), and Zap1p, a transcriptional repressor of genes required for biofilm extracellular matrix production (72). As with bacteria, signaling molecules associated with fungal quorum sensing, such as tyrosol and farnesol, modulate biofilm formation in *C. albicans* (73). Tyrosol enhances early biofilm events including adhesion and filamentation (73). In contrast, farnesol is secreted later in biofilm development and inhibits adhesion, possibly facilitating biofilm dispersion (73).

Therapeutics Targeting Fungal Biofilms

A number of recent studies have suggested that, as with bacteria, targeting biofilm formation by fungi may be an attractive therapeutic strategy. Echinocandins inhibit Fks1p, the β-1,3-glucan synthase, and exhibit anti-biofilm activity *in vitro* and in animal models of catheter-associated *C. albicans* infection (74,

75). It has been hypothesized that the anti-biofilm activity of these agents may underlie their success in clinical trials for the treatment of *Candida* infections. These agents do not exhibit anti-biofilm activity against *A. fumigatus*, as they do not influence the synthesis of GAG. The use of therapeutic enzymes that degrade the biofilm matrix has been explored in both *C. albicans* and *A. fumigatus*. Therapy with recombinant DNase reduces *C. albicans* biofilm biomass and enhances the activity of amphotericin B, but not fluconazole, *in vitro* (76, 77). Similarly, recombinant DNase was found to disrupt *A. fumigatus* biofilms and to enhance the activity of several antifungal agents *in vitro* (78). No studies in animal models evaluating the effects of DNase on antifungal susceptibility have been reported to date. However, β-glucanase therapy enhanced the activity of neutrophils and antifungals against *C. albicans* biofilms both *in vitro* and in a rat catheter model of infection (6, 68), thus providing evidence that biofilm-degrading enzymes can be effective *in vivo*. The use of hydrolytic enzymes targeting GAG has not yet been explored as an anti-biofilm strategy against *A. fumigatus*; however, Sph3 has been demonstrated to hydrolyze both purified and cell wall-associated GAG (15), suggesting that this may be a promising avenue to explore in the future.

Conclusions

Biofilm formation by fungi is emerging as an important factor in the pathogenesis of human fungal disease, and as an important factor when considering the choice of therapeutic strategies for these diseases. Despite their evolutionary distance from bacteria, fungi utilize many of the same mechanisms during the synthesis and modification of biofilm exopolysaccharides as bacteria. Fungal glycans play a similar role in mediating protection against immune defenses and antimicrobial agents such as bacteria exopolysaccharides. The development of new anti-biofilm therapeutic agents holds great potential as a strategy for the treatment of fungal infections, either alone, or in combination with conventional antifungals.

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