The Cell Biology of Fission Yeast Septation

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SUMMARY

In animal cells, cytokinesis requires the formation of a cleavage furrow that divides the cell into two daughter cells. Furrow formation is achieved by constriction of an actomyosin ring that invaginates the plasma membrane. However, fungal cells contain a rigid extracellular cell wall surrounding the plasma membrane; thus, fungal cytokinesis also requires the formation of a special septum wall structure between the dividing cells. The septum biosynthesis must be strictly coordinated with the deposition of new plasma membrane material and actomyosin ring closure and must occur in such a way that no breach in the cell wall occurs at any time. Because of the high turgor pressure in the fungal cell, even a minor local defect might lead to cell lysis and death. Here we review our knowledge of the septum structure in the fission yeast *Schizosaccharomyces pombe* and of the recent advances in our understanding of the relationship between septum biosynthesis and actomyosin ring constriction and how the two collaborate to build a cross-walled septum able to support the high turgor pressure of the cell. In addition, we discuss the importance of the septum biosynthesis for the steady ingression of the cleavage furrow.

INTRODUCTION

Cytokinesis is the final stage of the eukaryotic cell cycle, where, after spindle disassembly and mitotic exit, the formation of a cleavage furrow results in the separation of the cell into two new and identical cells. In animal cells, furrow formation requires the formation, maintenance, and closure of an actomyosin ring (AMR), coupled with the deposition of new plasma membrane material. Fungal cells are surrounded by a rigid cell wall exoskel-

eton; thus, AMR contraction is tightly coordinated with the biogenesis of a special wall structure named the division septum (1).

Animal cells contain an external structure made of polysaccharides and proteins termed the extracellular matrix. Although the extracellular matrix is not a rigid structure and does not provide osmotic support, it is considered the functional equivalent of the cell wall, and both structures are essential for the cell (2–5). In addition, as in fungal cells, some extracellular matrix polymers are also important for cytokinesis (6–9).

The last step of cytokinesis is the cell separation resulting from controlled and specific cell wall and septum degradation. Correct septum formation and, especially, cell separation are critical processes for cell integrity and survival (8, 10, 11). One of the main reasons for studying the cell wall and the septum structures is that the cell wall confers shape to the cell in a constantly changing pattern, thus serving as a good model for morphogenesis at the molecular level (12). Because of its highly regular and simple rod shape and growth patterns, the fission yeast *Schizosaccharomyces pombe* has been widely used as a model organism for the study of eukaryotic cytokinesis and morphogenesis (13). Here we provide an overview of how the septum structure is built in coordination with AMR closure and plasma membrane ingression in fission

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yeast, and we discuss the contribution of the septum synthesis to cleavage furrow ingression.

CELL WALL AND SEPTUM IN FISSION YEAST

Cell Wall Composition and Structure

The fission yeast cell wall consists mainly of polysaccharides made up of three different sugars: glucose, mannose, and galactose. Two glucose polysaccharides are the major structural components of the cell wall: $\beta(1,3)$ -D-glucan, with 14% $\beta(1,6)$ branches (B-BG), constitutes 48% to 54% of the total cell wall polysaccharides; and linear $\alpha(1,3)$ -D-glucan, with 7% $\alpha(1,4)$ bonds located at the reducing end of each chain, constitutes 28% to 32% of the cell wall (14–17).

Additionally, a special linear $\beta(1,3)$ -D-glucan (L-BG) with no $\beta(1,6)$ branches has been detected (18, 19). Another polysaccharide, a highly branched $\beta(1,6)$ -D-glucan with 75% $\beta(1,3)$ bonds, represents only 5% to 10% of the total and could be important for cross-linking the different polysaccharides of the cell wall. Because of the abundance of both types of glucose links, it is also called a diglucan (20, 21). The nonstructural galactomannan is linked to proteins to form the glycoprotein layer that is composed of an $\alpha(1,6)$ -D-mannose backbone with branches formed by $\alpha(1,2)$ - or $\alpha(1,3)$ -linked D-mannoses containing galactose units at the terminal non-reducing-end positions, constituting 9% to 14% of the cell wall (14, 22, 23). Most proteins of the cell wall are water or detergent soluble and are secreted into the medium. A few cell wall proteins are covalently linked to polysaccharides, forming two groups: proteins covalently attached to $\beta(1,3)$ -D-glucan (PIR proteins) through an alkali-labile glutamine residue and proteins covalently attached by a glycosylphosphatidylinositol (GPI) anchor to the $\beta(1,6)$ -D-glucan, which can be removed by glucanase treatment. Two PIR-type and 33 hypothetical GPI proteins encoded by the *S. pombe* genome have been previously described (24, 25). In contrast to most fungi, no chitin has been detected in the cell wall of vegetative cells in fission yeast (26, 27).

Electron microscopy of the cell wall shows a three-layer structure with two electron-dense layers separated by a nondense layer (8, 28–31). Immunoelectron microscopy, using specific lectins or antibodies, helped to define the organization of the different polysaccharides in the cell wall (19, 32–34). Galactomannan has been localized to the outer and inner sides of the cell wall (23). The nondense layer is mainly formed by B-BG, with the $\beta(1,6)$ -D-glucan close to the outer galactomannan external layer, a finding that led to the proposal that a $\beta(1,6)$ -D-glucan function connects the external surface proteins with the remaining cell wall polysaccharides (15, 19, 21). High-resolution electron microscopy analysis of the cell wall in regenerating protoplasts provided important information about the birth and formation of the cell wall ultrastructure. First, it is detected as a tangled network of $\beta(1,3)$ -D-glucan fibrils enveloping the spherical protoplast. Next, the fibrous network evolves and the protoplast cells acquire a more elongated shape, resulting in bundles and ribbons of $\beta(1,3)$ -D-glucan surrounded by galactomannan particles (35, 36). Mature $\alpha(1,3)$ -D-glucan has not been well detected by immunoelectron microscopy, but functional and structural analysis in the absence of Ags1 indicates that it is localized with the B-BG in the less-electron-dense region of the cell wall. This supports the idea that $\alpha(1,3)$ -D-glucan may be needed for the glucan bundle assembly in new cell wall formation (10, 33).

Septum Composition and Structure

Once the AMR is assembled in the cell middle during mitosis (1), coordinated and simultaneous AMR closure and septum deposition initiate after the mitotic exit (37). The septum is a three-layered structure consisting of a middle electron-transparent primary septum (PS) flanked by an electron-dense secondary septum (SS) on each side (Fig. 1, top). In contrast to the budding yeast *Saccharomyces cerevisiae*, where the SS appears to be assembled after the PS is completed (38), fission yeast septum grows by simultaneous synthesis of both the PS and the SS (8, 10). After septum completion, the septum thickness increases in a maturation process involving an additional round of SS synthesis. The PS is initiated in the inner surface of the cell wall. During centripetal septum growth, the PS progressively drills into the cell wall structure. The final septum displays a PS invading and reaching the middle of the cell wall and flanked by two triangular electron-dense structures termed “matériel triangulaire dense” (MTD; dense triangular material) whose function and composition are unknown and by a ring structure of dense material named the dense ring (DR) spanning the area from the PS and MTD to the outer cell wall surface (8, 10, 11, 29).

The fission yeast septum contains different essential glucans (Table 1). The $\beta(1,6)$ -D-glucan is localized in the SS; the L-BG is located and abundant in the PS; and B-BG and $\alpha(1,3)$ -D-glucan are located in both the PS and the SS (10, 18, 19). The L-BG is an essential component of the PS, but it is not the only polysaccharide of this structure, which also contains at least B-BG and $\alpha(1,3)$ -D-glucan. In fact, studies of L-BG depletion showed the presence of septa with disperse L-BGs unable to assemble a PS structure, showing that the L-BG is necessary but not sufficient for PS formation (18).

Although some studies have suggested the presence of glycoproteins in budding yeast (52–54), the outer glycoprotein layer is not detected either in the septum structure or in the cell wall surface of the ends generated after PS degradation and cell separation. However, since the cell wall of the new end originates with the SS, it is reasonable to believe that, once formed, the SS contains the glycoproteins responsible for new cell wall remodeling.

Cell Wall and Septum Architecture

Most of our knowledge about how all of these wall polysaccharides are linked to each other to form a robust network that preserves cell integrity comes from studies in budding yeast. Briefly, $\beta(1,6)$ -D-glucan is attached both to the nonreducing ends of $\beta(1,3)$ -D-glucan and to GPI mannoproteins through their glycosylphosphatidylinositol (GPI) anchor. PIR proteins are covalently linked to $\beta(1,3)$ -D-glucan through an alkali-labile glutamine bond (55). Only two PIR-type proteins have been described in *S. pombe*: Psu1, similar to *S. cerevisiae* SUN family proteins; and Asl1, related to proteins from *Aspergillus fumigatus* and *Ustilago maydis* (25). Chitin is a minor component of the budding yeast cell wall, but it is essential for cell integrity because of its function in cell division. This polysaccharide is attached either to $\beta(1,6)$ -D-glucan via a β -1,3-linked residue or to the nonreducing ends of $\beta(1,3)$ -D-glucan (2, 55). Analysis of the specific chitin attachment status at the lateral cell wall and at the mother-bud neck has recently led to the proposal that chitin in the cell wall is linked to the $\beta(1,6)$ -D-glucan, which is in turn attached to the nonreducing ends of $\beta(1,3)$ -

FIG 1 Models of the septation process and alternative septations of fission yeast. (Top) Septation in the wild-type cell. Simultaneous and coordinated syntheses (green arrow) of PS (red) and SS (green) form a three-layered septum. Septum maturation proceeds by PS anchorage into the cell wall (brown arrow) and, after septum completion, by a second round of SS synthesis. (Middle) Septation in the absence of Bgs1. The septum grows by successive SS depositions located parallel to the cell wall (orange arrow). The matériel triangulaire dense (MTD) changes to a septum medial position, forming a dotted line of matériel dense (MD) in the SS layers (18). (Bottom) Septation in the absence of Bgs4. AMR and septum are obliquely positioned in the middle of the cell. The septum grows as a weak, twisted, and misdirected PS (wavy arrow) whose formation is delayed and uncoupled from AMR and plasma membrane ingression (red arrow). After septum completion, the defective middle region is repaired with new a PS (orange arrow) and the PS base is retracted from the cell wall, leaving a wide region of dense material. PS, primary septum; SS, secondary septum. B-BG, branched $\beta(1,3)$ -D-glucan; L-BG, linear $\beta(1,3)$ -D-glucan; PS, primary septum; SS, secondary septum. Bar, 1 μm . (Adapted from reference 8.)

D-glucan, allowing cell wall growth. However, at the neck wall, chitin competes with $\beta(1,6)$ -D-glucan for the nonreducing ends of the $\beta(1,3)$ -D-glucan, and this linkage stops the neck wall growth during budding (2). In the fission yeast cell wall, glycoproteins might be integrated into the cell wall through linkages between mannose residues and $\alpha(1,3)$ -D-glucan fibrils (10). In addition, spherical mutants affected in the function of Bgs4 glucan synthase (GS) (see below) show a considerable reduction in the galactomannan content of the cell wall, releasing most of the glycoproteins to the growth medium, which points to the existence of a link between $\beta(1,3)$ -D-glucan and galactomannan (48, 56). Additionally, galactomannan could be bound to the glucan network through $\beta(1,6)$ -D-glucan, as is the case in the budding yeast cell wall.

Fission yeast PS does not contain chitin, while budding yeast PS appears to be mainly or exclusively composed of chitin. This PS chitin is found to be polydispersed but larger on average than that in the lateral cell wall. Most PS chitin is free, and about 14% is linked to $\beta(1,3)$ -D-glucan. There is no evidence regarding the function of this glucan cross-link, but an attractive supposition is that it may function by attaching the PS to the flanking SS, while the longer free chitin chains may well play a structural role (57).

It has been proposed that the association between chitin and glucans confers to the cell wall the mechanical strength needed to withstand the internal pressure (58–60). Given that chitin is ab-

sent in fission yeast, linkages between α - and β -glucans should be enough to confer mechanical strength to the septum and cell wall; thus, α - and β -glucans would have probably acquired multiple essential functions in the cell wall during the different stages of the cell cycle (Table 1 and below), as is the case for chitin in budding yeast (2, 8, 10, 18).

SYNTHESIS OF THE FISSION YEAST SEPTUM

As stated above, the fission yeast septum is mainly composed of essential α - and β -glucans. Although the $\beta(1,6)$ -D-glucan must be important to interconnect the wall polysaccharides, our knowledge about how it is synthesized and incorporated into the fission yeast cell wall is very limited (61). To date, most of the genes implicated in the synthesis of $\beta(1,6)$ -D-glucan have been identified in *S. cerevisiae*. The site of $\beta(1,6)$ -D-glucan synthesis has been controversial for many years, although some authors have suggested that biosynthesis of this polymer could start in the endoplasmic reticulum, whereas others have suggested that $\beta(1,6)$ -D-glucan, like $\beta(1,3)$ -D-glucan (at least, most of it), may be synthesized at the plasma membrane (62–64). In *S. pombe*, an immunoelectron microscopy analysis showed particles of $\beta(1,6)$ -D-glucan associated with the Golgi apparatus (Table 1), suggesting that biosynthesis of this polymer progresses in the Golgi apparatus and is completed at the cell surface (19).

TABLE 1 Localization and function of the cell wall polysaccharides during cell growth and cytokinesis

Cell wall polysaccharide	Synthases	Immunoelectron microscopy location	Cell wall structure	Polysaccharide function	Reference(s)
$\alpha(1,3)$ -D-Glucan	Ags1/Mok1	Unknown	Cell wall Primary septum Secondary septum	Secondary septum formation Cell integrity Primary septum strength Adhesion between primary and secondary septum Gradual cell separation	10, 39, 40
Linear $\beta(1,3)$ -D-glucan	Bgs1/Cps1/Drc1	Cell wall Primary septum	Cell wall Primary septum	Polar growth Cell separation Primary septum formation Stable anchorage of the actomyosin ring in the cell middle ^a Actomyosin ring integrity during septation ^a Location of Ags1 and Bgs4 Cleavage furrow formation	18, 19, 41, 42, 43, 44, 45, 46, 47
Branched $\beta(1,3)$ -D-glucan	Bgs4/Cwg1/ Orb11/Pbr1/Sph1	Cell wall Primary septum Secondary septum	Cell wall Primary septum Secondary septum	Polar growth Secondary septum formation Cell integrity Septum strength Stable anchorage of the actomyosin ring in the cell middle Coupling of septum synthesis progression with actomyosin ring constriction and septum membrane ingression	8, 19, 48, 49, 50–51
Branched $\beta(1,6)$ -D-glucan	Unknown	Cell wall Secondary septum Golgi apparatus Vesicles	Unknown	Unknown	19

^a Whether the function is due to the protein or to the polysaccharide has yet to be experimentally demonstrated.

FIG 2 Model of the $\beta(1,3)$ -D-glucan synthesis activation. The enzyme $\beta(1,3)$ -D-glucan synthase, which uses UDP-glucose as a substrate, produces a new chain of linear $\beta(1,3)$ -D-glucan. The enzymatic complex, comprising at least two subunits, is located at the inner side of the plasma membrane. Biochemical studies have revealed the fractionation of the enzyme into two components: the GTPase Rho1 is the regulatory subunit responsible for the activation of enzyme, while the catalytic subunit would be composed of Bgs1, Bgs2, Bgs3, and/or Bgs4. The exchange of GDP with GTP in Rho1 is shown in the cytoplasm, but it could take place at the plasma membrane. Glucose units (blue circles) are bound to UDP. A section of the cortical F-actin patches (red circles) around a plasma membrane invagination is shown.

$\beta(1,3)$ -D-Glucan Synthases

In fungal cells, the *in vitro* $\beta(1,3)$ -D-glucan synthase (GS) activity is responsible for the biosynthesis of short chains of linear $\beta(1,3)$ -D-glucan (Fig. 2). The GTPase Rho1 is an essential regulatory subunit of this activity (65–67). Four genes, *bgs1*⁺ to *bgs4*⁺, which encode four putative GS catalytic subunits, have been identified in fission yeast (Fig. 2); three of them (*bgs1*⁺, *bgs3*⁺, and *bgs4*⁺) are essential during vegetative growth, and the other (*bgs2*⁺) is essential during sexual differentiation for spore wall maturation. Bgs1, -3, and -4 localize to the AMR, septum, growing poles, and sites of wall synthesis during sexual differentiation (54, 68, 69). In contrast to what is seen in budding yeast and some other fungi, where Fks1 and Fks2 have redundant roles, fission yeast GS catalytic subunits display differential and essential nonoverlapping roles in the synthesis of different β -glucans during cell wall and septum assembly.

Bgs1. *bgs1*⁺ (also called *cps1*⁺ and *drc1*⁺) was first identified by complementation of the *cps1-12* mutant hypersensitive to a spindle poison. Interestingly, the fact that this mutant does not present a reduction in either cell wall β -glucan or *in vitro* GS activity suggested that Bgs1 might be responsible for the synthesis of a minor $\beta(1,3)$ -D-glucan. Additionally, the *cps1-12* mutant displays a multiseptated and branched phenotype, and thus it was proposed that Bgs1 could be a GS involved in cytokinesis, polarity, and cell wall morphogenesis (41). Two other mutants, *swl1-N12* (*cps1-N12*) and *drc1-191* (*cps1-191*), were described as forming a stable AMR but showing an inability to assemble the division septum, implicating Bgs1 in a septation checkpoint (42, 70). The phenotype of these mutants led to the proposal that Bgs1 could be

a GS essential for septum assembly. However, since there are no biochemical data supporting the proposed reduction of the septum $\beta(1,3)$ -D-glucan or the *in vitro* GS activity, it is unknown how these mutations compromise the biosynthetic activity of Bgs1. It has recently been reported that *cps1-191* allows the slow formation of a partial septum which is heterogeneously stained with the fluorochrome calcofluor white (CW), so it is possible that this mutated version of Bgs1 still maintains some residual activity at high temperatures (43, 71). The finding that Bgs1 was localized at the AMR, and that it was essential for cell survival, suggested that it was required for PS formation (44, 45). However, Bgs1 localizes not only to the AMR but also to the septum, growing poles, and sites of cell wall synthesis during sexual differentiation (44). In contrast to *cps1-N12* and *cps1-191* mutant cells, the depletion or absence of Bgs1 induces a phenotype of multiseptated cells that eventually die. Ultrastructural analysis of the septa formed in *bgs1* Δ cells from germinating *bgs1* Δ spores established that Bgs1 is responsible for the L-BG synthesis and PS formation (Fig. 1, middle) and that CW binds specifically and with high affinity to the L-BG of the PS (18). However, a strong depletion of Bgs1 after 60 h of *bgs1*⁺ repression still showed abundant septa with L-BG and, in fewer cases, with residual PS structures. This observation is even more surprising considering that the first 10 h of repression results in a more than 300-fold reduction in Bgs1 and that this repression continues to increase; however, L-BG and PS were still detected after 60 h. Although Bgs1 is ultimately responsible for L-BG synthesis, because *bgs1* Δ cells do not show any trace of L-BG and the corresponding PS, this observation supported the still unconfirmed possibility that Bgs1 is not the synthase but a subunit of the catalytic complex that is essential for activating and directing L-BG synthesis (18).

Bgs2. Bgs2 is essential during the sexual phase of the life cycle. The GS activity is reduced in sporulating homozygous *bgs2* Δ /*bgs2* Δ diploid cells (72). Bgs2 is observed around the spore periphery and is required for the correct assembly of the spore wall and survival (72, 73).

Bgs3. *bgs3*⁺ was identified as a suppressor of a mutant that shows hypersensitivity to the antifungal GS inhibitor echinocandin. Bgs3 is essential, although its function still remains unknown (74, 75).

Bgs4. *bgs4*⁺ (also called *cwg1*⁺, *orb11*⁺, *sph1*⁺, and *pbr1*⁺) encodes the only subunit that has been shown to form part of the GS enzyme. It is responsible for the synthesis of the cell wall B-BG (Fig. 1, bottom) and the major *in vitro* GS activity, and it is essential for the maintenance of cell shape and integrity during cell growth and for SS formation and correct PS completion during cytokinesis (8, 48–50, 76). In agreement with its role synthesizing the major B-BG, the only mutants of fission yeast identified to date that display reduced levels of cell wall β -glucan and GS activity or display resistance to the specific GS inhibitors (papulacandins, enfumafungin, and echinocandins) are due to point mutations in conserved and short regions (named hot spots of resistance) of the Bgs4 sequence (48, 50, 51, 77). The fact that a simple mutation of Bgs4, with Bgs1 and Bgs3 both being wild type, is able to confer resistance to the cell with respect to the specific GS inhibitors and the lack of isolated mutations in *bgs1*⁺ and *bgs3*⁺ that confer resistance to GS inhibitors indicate that the encoded Bgs1 and Bgs3 proteins are natural intrinsic resistant GS subunits (Fig. 3). In support of this notion, either the treatment with specific GS inhibitors or the absence of Bgs4 induces cell lysis and cytoplasmic

FIG 3 Protein sequence alignment of two conserved regions of Bgs1, Bgs2, Bgs3, and Bgs4 from *S. pombe*, Fks1 and Fks2 from *Saccharomyces cerevisiae* (Sc), Gsc1 (Fks1) from *Candida albicans* (Ca), and Fks1 and Fks2 from *Candida glabrata* (Cg). Mutations in the residues depicted in red confer resistance to echinocandins in *S. pombe*, *S. cerevisiae*, *C. albicans*, or *C. glabrata*, defining resistance hot spot 1 and hot spot 2. The Bgs4^{pbr1-8} mutation is located 4 amino acids N terminal from hot spot 1, increasing the cluster to a highly conserved 13-amino-acid hot spot (i.e., hot spot 1-1) of resistance to papulacandin, enfumafungin, and echinocandins. The Bgs4^{pbr1-6} change defines a new hot spot (i.e., hot spot 1-2) of resistance to the three antifungal families, located 48 amino acids C terminal from hot spot 1-1. (Adapted from reference 51 [copyright American Society for Biochemistry and Molecular Biology].)

release mainly during cell separation (8, 49, 51), whereas cell lysis is not observed in cells deprived of Bgs1 or Bgs3 (18, 75). Thus, it should be taken into account that the available GS inhibitors suppress only the GS activity due to Bgs4 but not the GS activity derived from Bgs1 or Bgs3 (51).

All the members of the family of fungal Bgs/Fks proteins and plant callose synthase CalS are large proteins (~200 kDa) with 15 to 16 predicted transmembrane domains divided into two hydrophobic regions and separated by three hydrophilic regions. Their central hydrophilic region displays a notable (>80%) degree of identity among all known Bgs/Fks/CalS proteins. This domain is thought to be located on the cytoplasmic face of the plasma membrane and to be essential for the function of the GS. Although it is believed that Bgs/Fks proteins are the catalytic subunits of the GS enzyme that synthesizes the $\beta(1,3)$ -D-glucan chains *in vivo*, and the results determined with different organisms strongly suggest this, none of these proteins contain the proposed UDP-glucose binding consensus (R/K)XGG of glycogen synthases in their protein sequence. In addition, the GS has not been purified from the plasma membrane to homogeneity and isolated, and therefore, the ability of reconstituted purified GS to synthesize *in vitro* $\beta(1,3)$ -D-glucan chains has not yet been demonstrated (44, 62, 78). Furthermore, the different mutations in the Fks1 sequence of budding yeast induce either a reduction or an increase in the levels of both $\beta(1,3)$ -D-glucan and $\beta(1,6)$ -D-glucan simultaneously (79). Therefore, other hypotheses about the function of Bgs should not be discarded. Fission yeast Bgs proteins could combine into different transmembrane pore complexes to synthesize and/or guide the chains of the different types of β -glucans along the plasma membrane to the periplasmic space. Therefore, to conclusively demonstrate that the Bgs/Fks proteins are the GS catalytic subunits and to definitively uncover the role of each Bgs protein specifically synthesizing a distinct β -D-glucan, additional knowledge from purified active membrane-bound complexes will be required.

$\alpha(1,3)$ -D-Glucan Synthase: Ags1/Mok1

Unlike the GS activity, an *in vitro* $\alpha(1,3)$ -D-glucan synthase activity has not yet been detected. Ags1/Mok1 is the putative $\alpha(1,3)$ -D-glucan synthase responsible for the synthesis of the cell wall $\alpha(1,3)$ -D-glucan and is essential for cell integrity (39, 40). Ags1 is predicted to be a large integral membrane protein of 272 kDa, with a cytoplasmic synthase domain, a hydrophobic region with multiple transmembrane domains, and an extracellular transglycosylase domain. The cytoplasmic synthase domain may add glucose residues to the nonreducing end of an $\alpha(1,3)$ -D-glucan chain, whereas the large extracellular N-terminal region may well function in cross-linking newly synthesized $\alpha(1,3)$ -D-glucan to other cell wall components (80). Like the Bgs proteins, Ags1 is found in the AMR, septum, growing poles (10, 34), and sites of wall synthesis during sexual differentiation and, together with Bgs4, is responsible for the assembly of the SS (10). Additionally, Ags1 grants to the PS the robustness needed to counteract the turgor pressure for a gradual cell separation (10). Fission yeast contains four additional Ags1 homologues (Mok11 to Mok14), which are detected only during sporulation (81). Ags1 orthologues are not found in budding yeasts but are widely extended in filamentous, dimorphic, and pathogenic fungi (82, 83).

FUNCTIONS OF THE WALL POLYSACCHARIDES DURING CELL DIVISION

Beyond the obvious structural role of polysaccharides in the assembly of the septum structures, recent studies have revealed new and important functions of the fission yeast polysaccharides attaching the AMR and coupling septum synthesis with cleavage furrow ingression (Table 1).

Anchorage and Maintenance of the AMR in the Cell Middle Plasma Membrane

To produce two identical daughter cells, the AMR must be positioned and kept in the cell middle before the septation onset. In

fission yeast, the nucleus and Mid1 mark the assembly of the AMR in the cell middle (1). However, during and after assembly, this ring must be spatially maintained for a proper cell division. Several reports have described findings indicating that the AMR slides sideways in round cells deprived of a cell wall, suggesting that, beyond the cell geometry, new cleavage furrow membranes or septum ingression might play a role in stabilizing and maintaining the ring in the cell middle (84–86). Something similar was previously described for *cps1-191* mutant cells depleted of either microtubules or Mid1 in the absence of septum deposition (87, 88). Since the AMR is fully assembled early at anaphase but starts constriction late after mitosis completion at telophase, it seems probable that the lateral cell wall, in combination with transmembranal linkages, might help to maintain the AMR in the cell middle before the glucan synthases localize to the cell middle and septation starts. In agreement, B-BG synthesized by Bgs4 is required to retain the AMR nodes and ring in the cell middle, before cleavage furrow ingression, suggesting that the AMR is linked to the extracellular cell wall through the plasma membrane (8). As stated above, Bgs1 has also been implicated in maintenance of the AMR position. The F-BAR protein Cdc15 would help to transfer Bgs1 (and probably the rest of the Bgs and Ags1 proteins) from the Golgi apparatus to the plasma membrane (10, 46). Thus, when Bgs1 recruitment to the cell middle is delayed by the presence of a compromised Cdc15 function, the AMR slides away from the cell middle (46). As described for Ags1, this delay in Bgs1 recruitment could be explained by a general delay in AMR formation when Cdc15 function is reduced (1, 10, 89). Despite these observations, it is still unknown whether it is Bgs1 itself or its product that is responsible for the stable AMR maintenance in the cell middle and how this attachment is achieved. Recently, it has been reported that absence of paxillin Pxl1, a conserved ring protein that is required for AMR integrity and whose localization depends on the SH3 domain of Cdc15 (89–92), induces simultaneous Bgs1 sliding and AMR sliding from the cell middle, indicating that the mere presence of Bgs1 alone is not enough to stably maintain the ring location (43). In agreement, the *cps1-191* mutant, in which the function of Bgs1 is compromised, also displays AMR sliding (43, 46, 87, 88). The precise coincidence between the emergence of a PS structure and the arrest of AMR sliding suggests that the cleavage furrow of PS and membrane ingression might be required for the stable AMR maintenance in the cell middle (43). Interestingly, the combined reduction of Pxl1 function and Cdc15 function induces Bgs1 and AMR sliding even after activation of synthesis of L-BG material, which is deposited along the longitudinal axis of the cell without cleavage furrow formation, suggesting that cooperation between the two proteins is needed to coordinate simultaneous activation of Bgs1 GS function and AMR constriction (43).

Coupling Septum Synthesis with Cleavage Furrow Ingression

The AMR is required for the normal synthesis of the septum (93), and, conversely, septum $\beta(1,3)$ -D-glucans contribute to maintain the ring structure during septation. A reduction of Bgs1 function through the use of the *cps1-191* mutant induced the formation of disorganized rings, showing that the septum L-BG or Bgs1 is required to maintain a stable AMR structure during septum progression (43). Bgs1 also collaborates with Pxl1 in maintaining the AMR and allowing septum ingression. Thus, when Bgs1 is depleted in the absence of Pxl1, the AMR disassembles prematurely

and septum formation is abolished (43). The close correlation between AMR closure and septum synthesis has encumbered the elucidation of whether the AMR is really pulling the plasma membrane enveloping the septum wall (94). In this regard, it has been traditionally thought that, as in metazoans, the *S. pombe* AMR exerts the pulling force needed to invaginate the plasma membrane to form the cleavage furrow (95, 96). However, the frequent observation by electron microscopy of bent or misaligned growing septa in wild-type cells, together with the fact that AMR mutants are able to assemble septa, favored the hypothesis that the centripetal ingression of the growing septum wall would be able to push the septum plasma membrane (94). Recent studies have revealed that both septum synthesis and AMR contraction are required for the correct ingression of the cleavage furrow (8, 71). In agreement with the previous proposal (94), it has been reported that greatly advanced septa are slowly terminated in the presence of the actin-depolymerizing drug latrunculin A. This observation, together with results showing the reduced rates of septum ingression in Bgs1-deficient *cps1-191* mutant cells, led to the proposition that Bgs1-dependent L-BG synthesis provides the major force required for plasma membrane invagination and AMR closure (71, 97). However, this hypothesis seems to be directly contradictory of the fact that a reduction of the level of the major B-BG, synthesized by Bgs4 and present in both PS and SS, produces (i) misdirected septum ingression, indicative of a weak, relaxed, and larger AMR; and (ii) a separation between slower PS synthesis and faster ring and membrane ingression (Fig. 4). All of these facts suggest that cleavage furrow formation can progress without the proposed mechanical pushing force of the glucans synthesized in the septum edge and without the suggested pulling force of AMR contraction, just by addition of membrane vesicles to the edge of the septum membrane (8). However, the close relationship between the AMR structure and septum synthesis still makes drawing conclusions about the real contributions of both AMR constriction and PS synthesis to the force required for septum membrane ingression complicated. It should be emphasized that the AMR is dispensable only for the completion of largely advanced septa but not with respect to their ingression rates, which become reduced overall, or for the ingression of septum rudiments, which is totally absent (71, 97). The reason for the different types of ingression behavior of short septa in the presence of a defective ring is unknown, but, globally, all these results highlight the findings that septum synthesis and AMR closure are both required for the steady progression of the cleavage furrow and that the septum membrane deposition also plays an important role. In fact, AMR constriction occurs in spherical spheroplast cells devoid of a cell wall, but, in this instance, the AMR slides along the membrane as it is constricting (85, 98). The AMR structure is required to maintain the spatial curvature and homogeneous rate of septum ingression, suggesting that the activity of the septum glucan synthases might be mechanosensitive and coupled to AMR tension (99, 100), although the higher septum membrane ingression rate observed in the septa in the absence of B-BG and with the AMR devoid of tensile force contradicts this suggestion (8).

In this regard, the septa are formed during Bgs1 depletion in the absence of PS by the successive deposition of SS layers, parallel to the lateral cell wall (Fig. 1, middle), which somehow are guided by the AMR to complete an aberrant septum (18). Pxl1 collaborates with Bgs1 to delimitate the area of septum synthesis by restricting the locations of the synthases Ags1,

FIG 4 Cleavage furrow ingression uncoupled from misdirected PS synthesis. (A) Misdirected septum ingression and relaxed AMR closure in cells with reduced branched $\beta(1,3)$ -D-glucan. During misdirected ingression, the AMR (red) (top) stays attached to the septum membrane (green) (top). The relaxed AMR structure permits changes in the orientation of the PS ingression, detected by calcofluor staining (blue) (bottom). Simultaneously, the PS synthesis is delayed from the AMR and septum membrane ingressions, as detected by the deficient calcofluor staining in the septum edge. (B and C) The branched $\beta(1,3)$ -D-glucan synthesized by Bgs4 is essential for coupling PS ingression to AMR contraction and plasma membrane extension. Rigid and straight wild-type septum formation with simultaneous PS (red) and SS (green) synthesis from the start is shown at the left, and advanced AMR and septum membrane ingressions uncoupled from delayed PS synthesis in the absence of Bgs4 are shown at the right. (C) Model of advanced AMR and septum membrane ingressions uncoupled from delayed PS synthesis. A relaxed AMR devoid of tensile force causes misdirected septa. The septum membrane progresses without AMR constriction and septum synthesis forces. Bar, 1 μ m. (D) Rigid and straight PS synthesis coupled to AMR and septum membrane progression (top) and delayed PS synthesis uncoupled from advanced AMR and septum membrane ingression (bottom) can also be observed in wild-type cells. Bar, 0.5 μ m. (E) Ultra-low-temperature-and-low-voltage scanning electron microscopy (ULT-LVSEM) images of a growing septum showing coupled septum synthesis (left) or an extended invaginated septum membrane ahead of the incomplete septum (right) in wild-type cells. White (A) and red (C) arrows, misdirected AMR closure and septum ingression. Black arrows (B and D), AMR and septum membrane edge; arrowheads (B and D), PS edge; AMR, actomyosin ring; PM, septum plasma membrane; PS, primary septum; SS, secondary septum; S, septum. (Adapted from references 8 [A to C], 10 [D], and 32 [E].)

FIG 5 Side-explosive cell separation in the absence of Ags1 $\alpha(1,3)$ -D-glucan. (A) Cell separation in wild-type cells. A balance between the osmotic pressure that curves the SS (green) to the stable spherical conformation and the controlled degradation of the septum edging (lateral cell wall) and PS (red) ensures a symmetrical and steady separation. (B) Side-explosive cell separation in the absence of Ags1 $\alpha(1,3)$ -D-glucan. Asymmetrical septum-edging degradation and mechanical tearing of a weak PS (red) that cannot hold the turgor pressure leads to an instantaneous side-explosive separation and to adoption of a stable spherical conformation in both new ends. The cells stay attached by the septum edging of the lateral cell wall for the next cell cycle. CW, cell wall; F, fuse channel (fuscanal); FS, fission scar; ICW, remedial internal cell wall layer; MTD, materiel triangulaire dense; NE, new end; Pr, turgor pressure; PS, primary septum; RSS, remedial secondary septum; SE, septum edging; SS, secondary septum. (Adapted from reference 10.)

Bgs4, and (probably) Bgs3. Thus, septum and cleavage furrow formation in cells depleted of Bgs1 depends entirely on the presence of Pxl1 in the AMR (43). In focal adhesions, paxillin binds to transmembranal α -integrins, where it might work as a mechanosensor to reinforce the connections between the extracellular matrix and cytoskeleton (101). A plausible hypothesis would be that fission yeast Pxl1 transmits the AMR tension to activate the Bgs1 function in the membrane, which somehow would help to concentrate Ags1, Bgs3, and Bgs4 at the cell equator. In the absence of Bgs1 and Pxl1, there is no PS synthesis and the linkage between the plasma membrane and the AMR is broken, all of which would promote delocalization of Ags1, Bgs3, and Bgs4, leading to widespread SS synthesis and the absence of cleavage furrow ingression.

Gradual Cell Separation and Cell Integrity

Cell separation is the most critical period of the cell cycle. During this stage, the lateral cell wall surrounding the septum and followed by the PS must be degraded in a very precise and controlled process. During cell separation, the degrading PS must support the internal turgor pressure of the cell in order to permit a gradual curvature of the SS and to reach the most stable hemispherical conformation to give rise to the new ends of the separating cells.

The normal septum structure is able to withstand the mechanical force promoted by the internal turgor pressure (Fig. 5A), and this PS strength allows symmetrical and gradual PS degradation and coupled SS curvature (10, 11, 102). However, a reduction of the level of Ags1-synthesized α -glucan causes a side-explosive cell separation originated by an instantaneous tearing of the PS, giving rise to two sister cells with remnants of PS in both new ends (Fig. 5B). These phenotypes indicate that the $\alpha(1,3)$ -D-glucan grants to the PS the mechanical force required to maintain the internal pressure during cell separation (10). In some cases, only the new end of one cell exhibited PS traces after the side-explosive separation, suggesting a faulty connection between PS and SS. The mechanical force provided by the $\alpha(1,3)$ -D-glucan to the PS during cell abscission suggested the existence of linkages between $\alpha(1,3)$ -D-glucan and $\beta(1,3)$ -D-glucans, similarly to the proposed role of the chitin- $\beta(1,3)$ -D-glucan bonds found in the PS of budding yeast (57).

After the septum completion, additional synthesis thickens the SS, followed by the appearance of a new structure of dense material (termed “dense ring”) spanning the area from the external cell wall surface to the deeply anchored base of the PS (Fig. 6, top). Cell separation starts by a highly controlled degradation of this dense

FIG 6 Models of the cell separation process. (Top) Wild-type cell separation. Controlled cell wall DR and PS (red) degradation (arrow) and the osmotic pressure that curves the SS (green) to the stable conformation ensure a safe separation. (Middle) In the absence of Bgs1 and its L-BG, there is no septum degradation and cell separation. (Bottom) In the absence of Bgs4 and its B-BG, uncontrolled cell wall DR degradation leaves the plasma membrane exposed to the medium. The turgor pressure causes curved septa (orange arrow) oscillating according to the changes in internal pressure between sister cells. The turgor pressure then generates the plasma membrane rupture and cytoplasm release to the medium. B-BG, branched $\beta(1,3)$ -D-glucan; DR, dense ring; F, fuscannel; FS, fission scar; L-BG, linear $\beta(1,3)$ -D-glucan; MD, matériel dense; MTD, matériel triangulaire dense; Pr, turgor pressure; PS, primary septum; SS, secondary septum. Bar, 1 μm . (Adapted from reference 8.)

ring, superseded by the gradual and specific degradation of the PS (8). Bgs4 depletion induces the formation of a thick and diffuse dense ring structure, causing uncontrolled cell wall degradation that leaves the plasma membrane exposed to the surrounding medium. In the absence of this lateral cell wall at the start of cell separation, the internal turgor pressure causes the rupture of the plasma membrane and release of the cytoplasmic content (Fig. 6, bottom). This observation showed that Bgs4 is essential for cell integrity, as it is required for the correct synthesis of the cell wall surrounding the PS and compensates for an excess of cell wall degradation during cell separation (8, 49). Ags1 is also essential for cell integrity in that Ags1-depleted cells also display a phenotype of cell lysis at the separation onset, suggesting that Ags1 and Bgs4 cooperate to ensure safe cell separation and, thus, the integrity of the cell (10).

CONCLUSION

We have just begun to understand the relationship between the machinery of cell wall synthesis and the formation of the cleavage furrow. Cell wall B-BG must be connected to the AMR through the plasma membrane, and this probably involves one or several membrane proteins able to connect the periplasmic space with the cytoplasm. The AMR defects in Bgs4-depleted cells are observed before the Bgs and Ags1 proteins are recruited to the cell middle, indicating that other transmembrane proteins might play a role

in this connection. Together with B-BG, α -glucan is the additional main structural polymer in the cell wall. Thus, it will be interesting to analyze whether this polysaccharide collaborates with B-BG to anchor the AMR during cytokinesis. Bgs1 must also have a role maintaining the AMR in the cell middle. Although it is unknown at present how this is accomplished, it may well involve either the function of Bgs1 and its GS activity or the new L-BG chains synthesized by Bgs1. The fact that the AMR slides away in *cps1-191* mutants suggests that Bgs1 activity could be important to anchor the ring. However, it is unknown whether *cps1-191* mutation truly compromises Bgs1 GS activity and/or L-BG synthesis. The isolation of new conditional mutants that are able to localize to the sites of active growth but that remain inactive, displaying the same phenotypes as those of Bgs1-depleted cells, would help to decipher the proposed function of the L-BG in anchoring the AMR and recruiting the other synthases.

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