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, and 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 conducted in such a way that no breach in the cell wall occurs at any time. Because of the high turgor pressure of the fungal cell, even a minor local defect may lead to cell lysis and death. Here we review our knowledge of the septum structure in the fission yeast *Schizosaccharomyces pombe*, and the recent advances about the relationship between septum biosynthesis and actomyosin ring constriction, and how both collaborate to build a cross-walled septum able to support the high turgor pressure of the cell. Besides, 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 separates 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 exoskeleton, thus AMR contraction is tightly coordinated with the biogenesis of a special wall structure named division septum (1).

Animal cells contain an external structure made of polysaccharides and proteins termed 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, like in fungal cells, some extracellular matrix polymers are also important for cytokinesis (6-9).

The last step of cytokinesis is the cell separation by controlled and specific cell wall and septum degradation. The correct septum formation and especially cell separation are critical processes for the cell integrity and survival (8, 10, 11). One of the main reasons for studying the cell wall and the septum structures is because 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 *S. 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 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

105 The fission yeast cell wall consists mainly of polysaccharides made up of three different
106 sugars: glucose, mannose and galactose. Two glucose polysaccharides are the major
107 structural components of the cell wall, β(1,3)-D-glucan with 14% of β(1,6) branches (B-
108 BG) constitutes 48-54% of the total cell wall polysaccharides, and linear α(1,3)-D-
109 glucan with 7% of α(1,4) bonds located at the reducing end of each chain, constitutes
110 28-32% of the cell wall (14-17).
111 Additionally, a special linear β(1,3)-D-glucan (L-BG) with no β(1,6) branches has been
112 detected (18, 19). Another polysaccharide, a highly branched β(1,6)-D-glucan with 75%
113 of β(1,3) bonds represents only 5-10%, and could be important for cross-linking the
114 different polysaccharides of the cell wall. Because of the abundance of both types of
115 glucose links it is also called diglucan (20, 21). The non-structural galactomannan is
116 linked to proteins to form the glycoproteins layer that is composed of an α(1,6)-D-
117 mannose backbone with branches formed by α(1,2)- or α(1,3)-linked D-mannoses
118 containing galactose units at the terminal non-reducing end positions, constituting 9-
119 14% of the cell wall (14, 22, 23). Most proteins of the cell wall are water- or detergent-
120 soluble and are secreted to the medium. Few cell wall proteins are covalently linked to
121 polysaccharides and form two groups: proteins covalently attached to β(1,3)-glucan
122 (PIR proteins) through an alkali-labile glutamine residue; and proteins covalently
123 attached by a GPI anchor to the β(1,6)-glucan that can be removed by glucanase
124 treatment. Two PIR-type and 33 hypothetical GPI proteins have been described in the
125 *S. pombe* genome (24, 25). In contrast to most fungi, no chitin has been detected in the
126 cell wall of vegetative cells in fission yeast (26, 27).
127 Electron microscopy of the cell wall shows a three-layer structure with two electron
128 dense layers separated by a non-dense layer (8, 28-31). Immunoelectron microscopy,
129 using specific lectins or antibodies, helped to define the organization of the different
130 polysaccharides in the cell wall (19, 32-34). Galactomannan has been localized to the
131 outer and inner sides of the cell wall (23). The non-dense layer is mainly formed by B-
132 BG, with the β(1,6)-D-glucan close to the outer galactomannan external layer, which

helped to propose a β-(1,6)-glucan function connecting 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. Firstly, it is detected as a tangled network of β(1,3)-D-glucan fibrils enveloping the spherical protoplast. Next, the fibrous network evolves and the protoplast cells acquire a more elongated shape, giving place to bundles and ribbons of β(1,3)-D-glucan surrounded by galactomannan particles (35, 36). Mature α(1,3)-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 α(1,3)-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 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, PS and 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 is progressively drilling 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 is unknown, and by a ring

160 structure of dense material named dense ring (DR) spanning from the PS and MTD to
161 the outer cell wall surface (8, 10, 11, 29).
162 The fission yeast septum contains different essential glucans (**Table 1**). The β(1,6)-D-
163 glucan is localized in the SS; L-BG is located and abundant in the PS; and B-BG and
164 α(1,3)-D-glucan are located in both PS and SS (10, 18, 19). The L-BG is an essential
165 component of the PS, but it is not the only polysaccharide of this structure, which also
166 contains at least B-BG and α(1,3)-D-glucan. In fact, studies of L-BG depletion showed
167 the presence of septa with disperse L-BG unable to assemble a PS structure, showing
168 that the L-BG is necessary but not sufficient for PS formation (18).
169 Although in budding yeast some studies suggest the presence of glycoproteins (39-41),
170 the outer glycoproteins layer is not detected either in the septum structure, or in the cell
171 wall surface of the ends generated after PS degradation and cell separation. However,
172 since the SS originates the cell wall of the new end, it is reasonable to believe that
173 once formed, the SS will contain the glycoproteins responsible for new cell wall
174 remodeling.
175
176 **Cell Wall and Septum Architecture**
177 Most of our knowledge about how all of these wall polysaccharides are linked to each
178 other to form a robust network that will preserve cell integrity, comes from studies in
179 budding yeast. Briefly, β(1,6)-D-glucan is attached to both the non-reducing ends of
180 β(1,3)-D-glucan, and to GPI mannoproteins through their glycosylphosphatidylinositol
181 (GPI) anchor. PIR proteins are covalently linked to β(1,3)-D-glucan through an alkali-
182 labile glutamine bond (42). Only two PIR-type proteins have been described in *S.*
183 *pombe*: Psu1, similar to *S. cerevisiae* SUN family proteins; and Asl1, related to proteins
184 from *Aspergillus fumigatus* and *Ustilago maydis* (25). Chitin is a minor component of
185 the budding yeast cell wall, but it is essential for cell integrity because of its function in
186 cell division. This polysaccharide is attached either to β(1,6)-D-glucan via a β-1,3-linked
187 residue, or to the non-reducing ends of β(1,3)-D-glucan (2, 42). Analysis of the specific

chitin attachment status at the lateral cell wall and at the mother-bud neck has recently led to propose that in the cell wall, chitin is linked to the β(1,6)-D-glucan that in turn is attached to the non-reducing ends of β(1,3)-D-glucan, allowing cell wall growth. However, at the neck wall, chitin competes with β(1,6)-D-glucan for the non-reducing ends of the β(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 α(1,3)-D-glucan fibrils (10). In addition, spherical mutants affected in the function of Bgs4 GS (see below) show a considerable reduction in the galactomannan content of the cell wall, releasing most of the glycoproteins to the growth medium, pointing to the existence of a link between β(1,3)-D-glucan and galactomannan (43, 44). Additionally, galactomannan could be bound to the glucan network through β(1,6)-D-glucan as is the case in the budding yeast cell wall.

Fission yeast PS does not contain chitin, while in budding yeast it appears to be exclusively or mainly composed of chitin. This PS chitin is found to be polydisperse, but larger on average than that in the lateral cell wall. Most PS chitin is free, and about 14% is linked to β(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 (45).

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 (46-48). Given that chitin is absent in fission yeast, linkages between α- and β-glucans should be enough to confer the mechanical strength to the septum and cell wall, and 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 β(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 (49). To date, most of the genes implicated in the synthesis of β(1,6)-D-glucan have been identified in *S. cerevisiae*. The site of β(1,6)-D-glucan synthesis has been controversial for many years although, it has been suggested that biosynthesis of this polymer could start in the endoplasmic reticulum, whereas other authors have suggested that β(1,6)-D-glucan, like β(1,3)-glucan, may be synthesized, at least most of it, at the plasma membrane (50-52). In *S. pombe*, an immuno-electron microscopy analysis showed particles of β(1,6)-D-glucan associated to the Golgi apparatus (**Table 1**), suggesting that biosynthesis of this polymer progresses in the Golgi and is completed at the cell surface (19).

β(1,3)-D-glucan Synthases

In fungal cells, the *in vitro* β(1,3)-D-glucan synthase (GS) activity is responsible for the biosynthesis of short chains of linear β(1,3)-D-glucan (**Fig. 2**). The GTPase Rho1 is an essential regulatory subunit of this activity (53-55). In fission yeast four genes, *bgs1*$^+$ to *bgs4*$^+$, which encode four putative GS catalytic subunits have been identified (**Fig. 2**), three of them (Bgs1, 3 and 4) being essential during vegetative growth, and the last one (Bgs2), being 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 (41, 56, 57). 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 non-overlapping roles in the synthesis of different β-glucans during cell wall and septum assembly:

(i) Bgs1

bgs1^{+}/*cps1*$^{+}$/*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 β(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 (58). Two other mutants, *swl1-N12* (*cps1-N12*) and *drc1-191* (*cps1-191*), were described as forming a stable AMR, but unable to assemble the division septum, implicating Bgs1 in a septation checkpoint (59, 60). The phenotype of these mutants led to the proposal that Bgs1 could be a GS essential for septum assembly. However, since there are not biochemical data supporting the proposed reduction of the septum β(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 (61, 62). 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 (63, 64). However, Bgs1 localizes not only to the AMR, but also to the septum, growing poles and sites of cell wall synthesis during sexual differentiation (63). 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 hours 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 hours of repression generates a more than 300-fold reduction in Bgs1, and that this

repression continues to increase; however, after 60 hours L-BG and PS are still detected. 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 unsolved possibility that Bgs1 is not the synthase, but a subunit of the catalytic complex essential to activate and direct L-BG synthesis (18).

(ii) 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 (65). Bgs2 is observed around the spore periphery and is required for the correct assembly of the spore wall and survival (65, 66).

(iii) 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 (67, 68).

(iv) Bgs4

bgs4^{+}/*cwg1*$^{+}$/*orb11*$^{+}$/*sph1*$^{+}$/*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, 43, 69-71). In agreement with its role synthesizing the major B-BG, to date the only identified mutants of fission yeast that display reduced levels of cell wall β-glucan and GS activity, or resistance to the specific GS inhibitors (papulacandins, enfumafungin and echinocandins) are due to point mutations in the Bgs4 sequence (43, 71-73). The fact that the simple mutation of Bgs4, with Bgs1 and Bgs3 both being wild type, is able to confer resistance to the cell to the specific GS

inhibitors, and the lack of isolated mutations that confer resistance to GS inhibitors in *bgs1*$^{+}$ and *bgs3*$^{+}$ indicates that the encoded proteins Bgs1 and Bgs3 are natural intrinsic resistant GS subunits (**Fig. 3**). In support of this, either the treatment with specific GS inhibitors or the absence of Bgs4 induce cell lysis and cytoplasmic release mainly during cell separation (8, 69, 73), whereas cell lysis is not observed in cells deprived of Bgs1 or Bgs3 (18, 68). Thus, it should be taken into account that the available GS inhibitors suppress only the GS activity due to Bgs4, but not of those derived from Bgs1 or Bgs3 (73).

All the family of fungal Bgs/Fks and plant callose synthase CalS, are large proteins (~200 KDa) with 15-16 predicted transmembranal domains divided into two hydrophobic regions and separated by three hydrophilic regions. Their central hydrophilic region displays a notable degree of identity (> 80%) 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 β(1,3)-D-glucan chains *in vivo*, and the observations depicted in 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* β(1,3)-D-glucan chains has not yet been demonstrated (50, 63, 74). Furthermore, the different mutations in the Fks1 sequence of budding yeast induce either a reduction or an increase of both β(1,3)-D-glucan and β(1,6)-D-glucan simultaneously (75). 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 undoubtedly demonstrate that 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.

α(1,3)-D-glucan Synthase: Ags1/Mok1

Unlike the GS activity, an *in vitro* α(1,3)-D-glucan synthase activity has not yet been detected. Ags1/Mok1 is the putative α(1,3)-D-glucan synthase responsible for the synthesis of the cell wall α(1,3)-D-glucan and is essential for cell integrity (76, 77). 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 non-reducing end of an α(1,3)-glucan glucan chain, whereas the large extracellular N-terminal region may well function in cross-linking newly synthesized α(1,3)-glucan to other cell wall components (78). 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 (Mok11–14), which are only detected during sporulation (79). Ags1 orthologues are not found in budding yeasts but are widely extended in filamentous, dimorphic and pathogenic fungi (80, 81).

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 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 (82-84). Something similar was depicted for *cps1-191* mutant cells depleted of either microtubules or Mid1 in the absence of septum deposition (85, 86). 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 Bgs and Ags1 proteins) from the Golgi apparatus to the plasma membrane (10, 87). 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 (87). 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, 88). Despite these observations, it is still unknown whether Bgs1 itself or its product, 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 required for AMR integrity and whose localization depends on the SH3 domain of Cdc15 (89-91), induces simultaneous Bgs1

and AMR sliding from the cell middle, indicating that the mere presence of Bgs1 alone is not enough to stably maintain the ring location (61). In agreement, the *cps1-191* mutant, in which the function of Bgs1 is compromised, also displays AMR sliding (61, 85-87). 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 (61). Interestingly, the combined reduction of Pxl1 and Cdc15 function induces Bgs1 and AMR sliding even after activation of synthesis of a L-BG material, which is deposited along the longitudinal axis of the cell without cleavage furrow formation, suggesting that a cooperation between both proteins is needed to coordinate simultaneous activation of Bgs1 GS function with AMR constriction (61).

Coupling Septum Synthesis with Cleavage Furrow Ingression

The AMR is required for the normal synthesis of the septum (92) and conversely, septum β(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 (61). Bgs1 also collaborates with Pxl1 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 (61). 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 (93). 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 (94, 95). 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, favoured the hypothesis that the centripetal ingression of the growing

412 septum wall could be able to push the septum plasma membrane (93). Recent studies
413 have revealed that both septum synthesis and AMR contraction are required for the
414 correct ingression of the cleavage furrow (8, 62). In agreement with the previous
415 proposal (93), it has been depicted that largely advanced septa are slowly terminated
416 in the presence of the actin depolymerizing drug latrunculin A. This observation,
417 together with the reduced rates of septum ingression in Bgs1-deficient *cps1-191*
418 mutant cells, led to the proposition that Bgs1-dependent L-BG synthesis provides the
419 major force required for plasma membrane invagination and AMR closure (62, 96).
420 However, this hypothesis seems to be directly contradictory with the fact that a
421 reduction of the major B-BG, synthesized by Bgs4 and present in both PS and SS,
422 produces i) misdirected septum ingression, indicative of a weak, relaxed and larger
423 AMR; and ii) a separation between a slower PS synthesis, and a faster ring and
424 membrane ingression (**Fig. 4**). All of which suggests that cleavage furrow formation
425 can progress without the proposed mechanical pushing force of the glucans
426 synthesized in the septum edge and without the suggested pulling force of AMR
427 contraction, just by addition of membrane vesicles to the edge of the septum
428 membrane (8). However, the close relationship between the AMR structure and the
429 septum synthesis still makes drawing conclusions about the real contributions of both
430 AMR constriction and PS synthesis to the force required for septum membrane
431 ingression complicated. It should be emphasized that the AMR is only dispensable for
432 the completion of largely advanced septa, but not for their ingression rates, which
433 overall become reduced, or for the ingression of septum rudiments, which is totally
434 absent (62, 96). The reason for the different ingression behavior of short septa in the
435 presence of a defective ring is unknown, but globally all these results highlight that
436 septum synthesis and AMR closure are both required for the steady progression of the
437 cleavage furrow and that the septum membrane deposition also plays an important
438 role. In fact, AMR constriction occurs in spherical spheroplast cells devoid of a cell wall,
439 but in this instance the AMR slides along the membrane as it is constricting (83, 97).

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 (98, 99). Although, the faster septum membrane ingression rate observed in the septa in the absence of B-BG and with the AMR devoid of tensile force, opposes this suggestion (8).

In this regard, during Bgs1 depletion the septa are formed 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 location of the synthases Ags1, Bgs4, and probably Bgs3. Thus, septum and cleavage furrow formation in cells depleted of Bgs1 depend entirely on the presence of Pxl1 in the AMR (61). In focal adhesions, paxillin binds to transmembranal α-integrins, where it might work as a mechanosensor to reinforce the connections between extracellular matrix and cytoskeleton (100). An plausible hypothesis would be that fission yeast Pxl1 might transmit the AMR tension to activate the Bgs1 function in the membrane, which somehow would help to concentrate Ags1, Bgs3 and Bgs4 in 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 Ags1, Bgs3 and Bgs4 delocalization, leading to widespread SS synthesis and 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

468 mechanical force promoted by the internal turgor pressure (**Fig. 5A**), and this PS
469 strength allows a symmetrical and gradual PS degradation and coupled SS curvature
470 (10, 11, 101). However, a reduction of Ags1-synthetized α-glucan causes a side-
471 explosive cell separation originated by an instantaneous tearing of the PS, giving rise
472 to two sister cells with remnants of PS in both new ends (**Fig. 5B)**. These phenotypes
473 indicate that the α(1,3)-D-glucan grants to the PS the mechanical force required to hold
474 up the internal pressure during cell separation (10). In some cases, only the new end of
475 one cell exhibited PS traces after the side-explosive separation, suggesting a faulty
476 connection between PS and SS. The mechanical force provided by the α(1,3)-D-glucan
477 to the PS during cell abscission suggested the existence of linkages between α(1,3)-D-
478 glucan and β(1,3)-D-glucans, similarly to the proposed role of the chitin–β(1,3)-D-
479 glucan bonds found in the PS of budding yeast (45).
480 After the septum completion, additional synthesis thickens the SS, followed by the
481 appearance of a new structure of dense material termed dense ring spanning from the
482 external cell wall surface to the deeply anchored base of the PS (**Fig. 6**, top). Cell
483 separation starts by a highly controlled degradation of this dense ring, superseded by
484 the gradual and specific degradation of the PS (8). Bgs4 depletion induces the
485 formation of a tick and diffuse dense ring structure, causing an uncontrolled cell wall
486 degradation that leaves the plasma membrane exposed to the surrounding medium. In
487 the absence of this lateral cell wall at the start of cell separation, the internal turgor
488 pressure causes the rupture of the plasma membrane and release of the cytoplasmic
489 content (**Fig. 6**, bottom). This observation showed that Bgs4 is essential for cell
490 integrity, as it is required for the correct synthesis of the cell wall surrounding the PS
491 and compensates for an excess of cell wall degradation during cell separation (8, 69).
492 Ags1 is also essential for cell integrity in the way that Ags1-depleted cells also display
493 a phenotype of cell lysis at the separation onset, suggesting that both Ags1 and Bgs4
494 cooperate ensuring a safe cell separation, and thus the cell integrity (10).
495

CONCLUSSION

We have just started 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 transmembranal 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 at present it is unknown 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 in *cps1-191* mutants the AMR slides away, suggests that Bgs1 activity could be important to anchor the ring. However, it is unknown whether *cps1-191* mutation is really compromising Bgs1 GS activity and/or L-BG synthesis. The isolation of new conditional mutants able to localize to the sites of active growth, but remaining inactive, displaying the same phenotypes as that 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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FIGURE LEGENDS

Figure 1. Models of the septation process and alternative septations of fission yeast. Top: Septation in the wild type cell. Simultaneous and coordinated synthesis (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 parallel to the cell wall (orange arrow). The MTD changes to a septum medial position, forming a dotted line of MD in the SS layers (18). Bottom: Septation in the absence of Bgs4. AMR and septum are obliquely positioned in the cell middle. The septum grows as a weak, twisted and misdirected PS (wavy arrow) that is delayed and uncoupled from AMR and plasma membrane ingression (red arrow). After septum completion, the defective middle region is repaired with new 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 β(1,3)-D-glucan; L-BG, linear β(1,3)-D-glucan; MD, matériel dense; MTD, matériel triangulaire dense; PS, primary septum; SS, secondary septum. Bar, 1 μm. Adapted from (8).

Figure 2. Model of the β(1,3)-D-glucan synthesis activation. The enzyme β(1,3)-D-glucan synthase, which uses UDP-glucose as a substrate, produces a new chain of linear β(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 allowed 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 by 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 are shown.

Figure 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 the resistance hot spot 1 and hot spot 2. The Bgs4$^{pbr1\text{-}8}$ mutation is located 4 amino acids N-terminal from hot spot 1, increasing the cluster to a 13-amino acid hot spot 1-1 of resistance to papulacandin, enfumafungin, and echinocandins. The Bgs4$^{pbr1\text{-}6}$ change defines a new hot spot 1-2 of resistance to the three antifungal families. Adapted from (73).

Figure 4. Cleavage furrow ingression uncoupled from misdirected PS synthesis.
(A) Misdirected septum ingression and relaxed AMR closure in cells with reduced branched β(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-C) The branched β(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 (left), and advanced AMR and septum membrane ingressions uncoupled from delayed PS synthesis in the absence of Bgs4 (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 μm. (D) Rigid and straight PS synthesis coupled to AMR and septum membrane progression (top) and delayed PS synthesis uncoupled from advanced AMR and

880 septum membrane ingression (bottom), can also be observed in wild type cells. Bar,
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883 septum (right) in wild type cells. White (A) and red (C) arrows, misdirected AMR closure
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885 Arrowhead (B, D), PS edge; AMR, actomyosin ring; PM, septum plasma membrane;
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888 Figure 5. Side-explosive cell separation in the absence of Ags1 α(1,3)-D-glucan.
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897 CW: cell wall; F: fuscannel; FS: fission scar; ICW: remedial internal cell wall layer;
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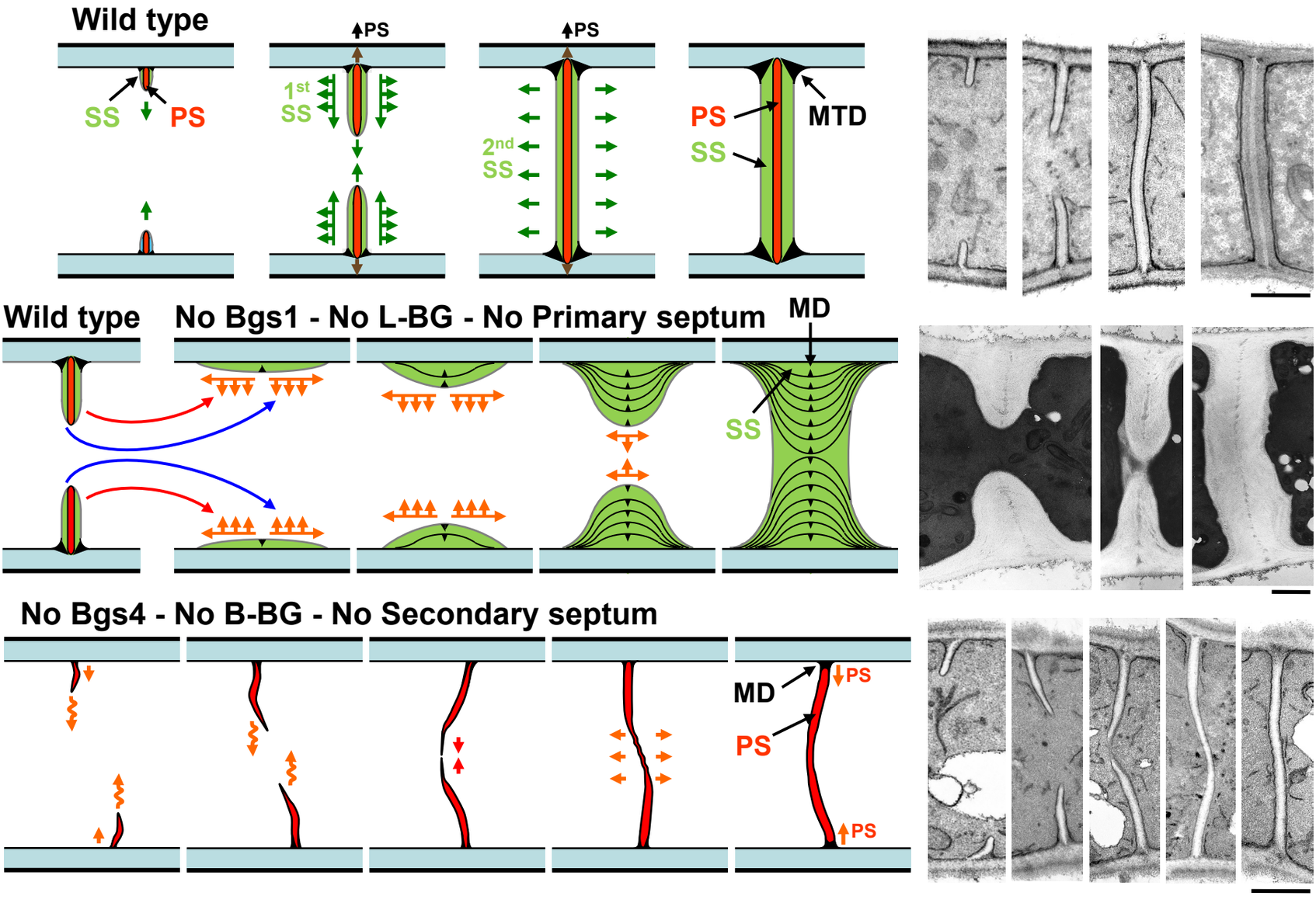
TABLE 1. Localization and function of the cell wall polysaccharides during cell growth and cytokinesis

Cell wall polysaccharide	Synthases	Immunoelectron microscopy localization	Cell wall structures	Polysaccharide functions	Reference(s)
α(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) (72) (73)
Linear β(1,3)-D-glucan	Bgs1/Cps1/Drc1	Cell wall Primary septum	Cell wall Primary septum	Polar growth Cell separation Primary septum formation [a]Stable anchorage of the actomyosin ring in the cell middle [a]Actomyosin ring integrity during septation Location of Ags1 and Bgs4 Cleavage furrow formation	(18) (19) (55) (56) (58) (60) (61) (83) (95)
Branched β(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 Couples septum synthesis progression with actomyosin ring constriction and septum membrane ingression	(8) (40) (66) (67) (68) (69) (70)
Branched β(1,6)-D-glucan	Unknown	Cell wall Secondary septum Golgi apparatus Vesicles	Unknown	Unknown	(19)

[a] It must be experimentally demonstrated whether the function is due to the protein or the polysaccharide



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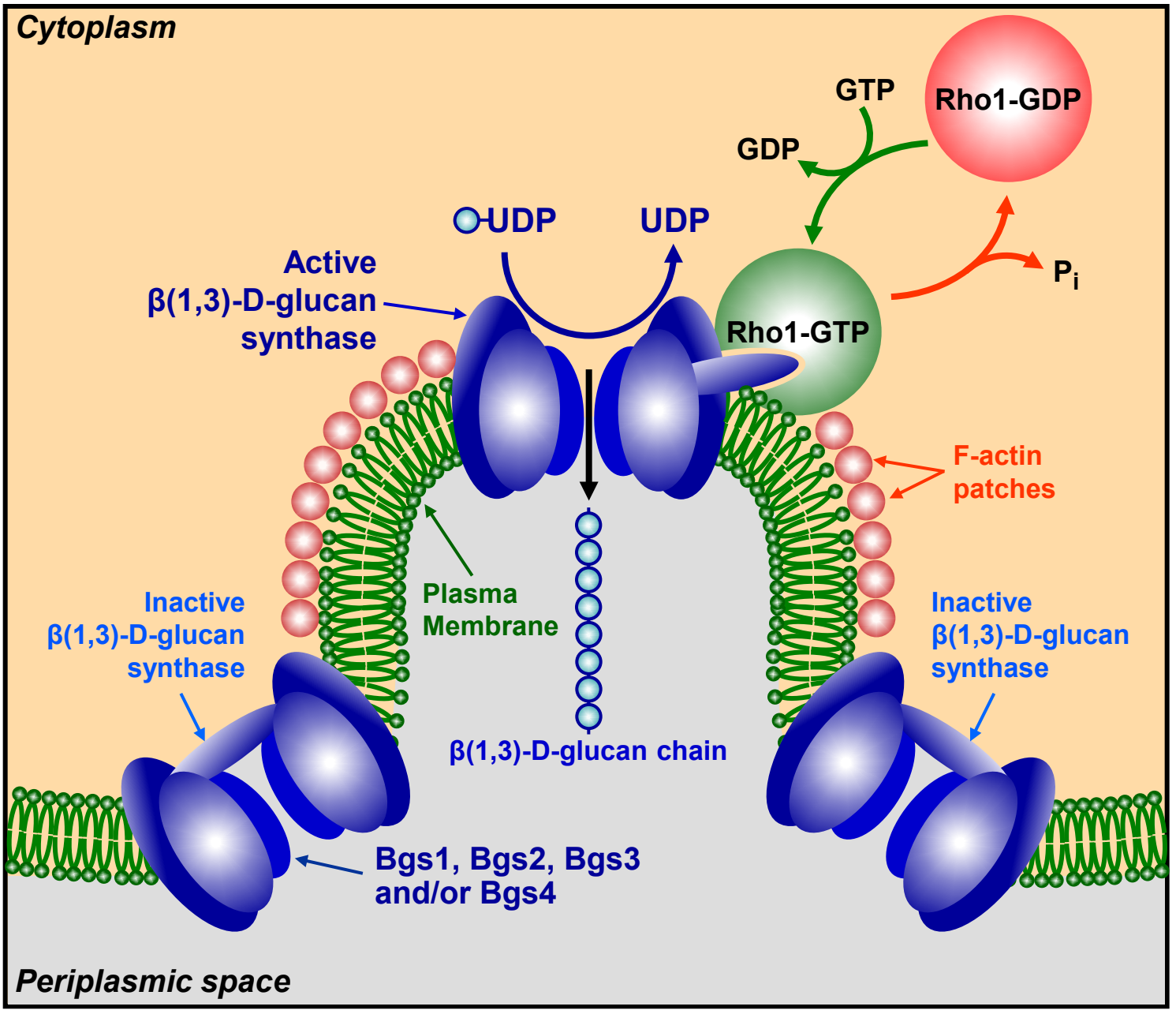

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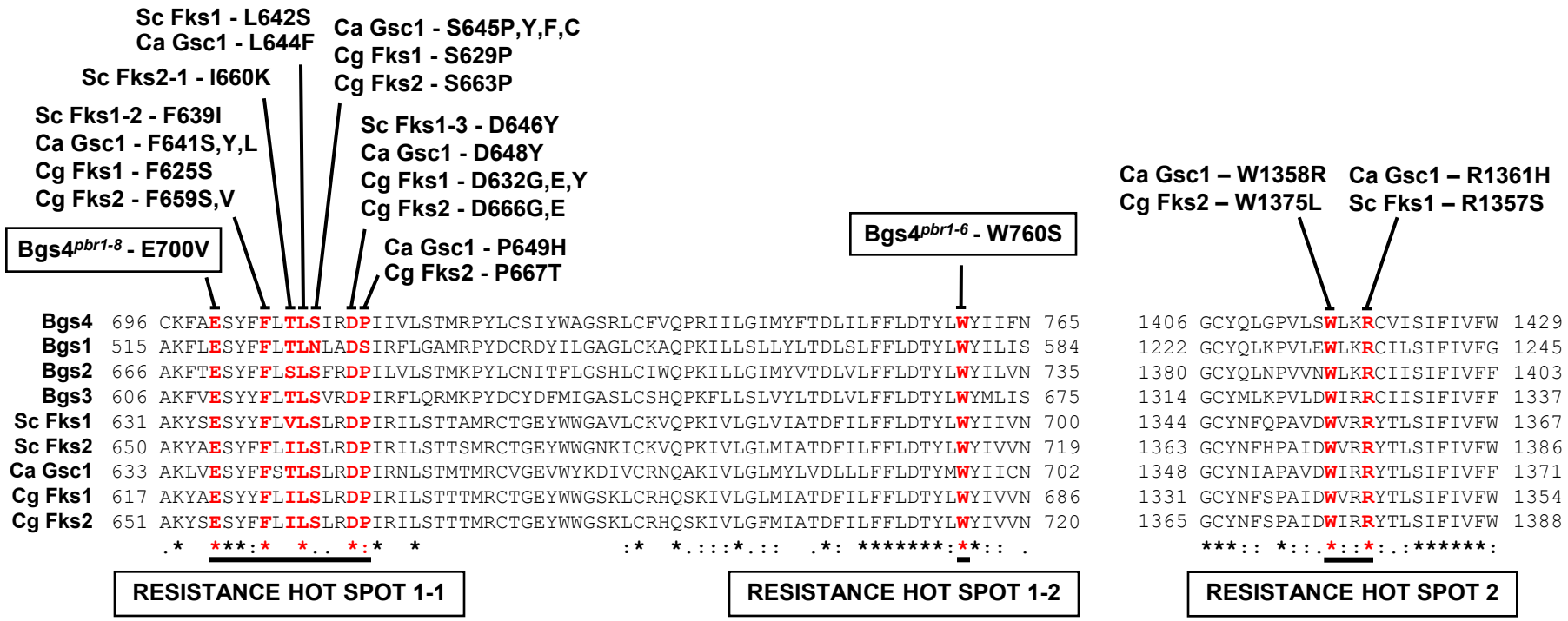

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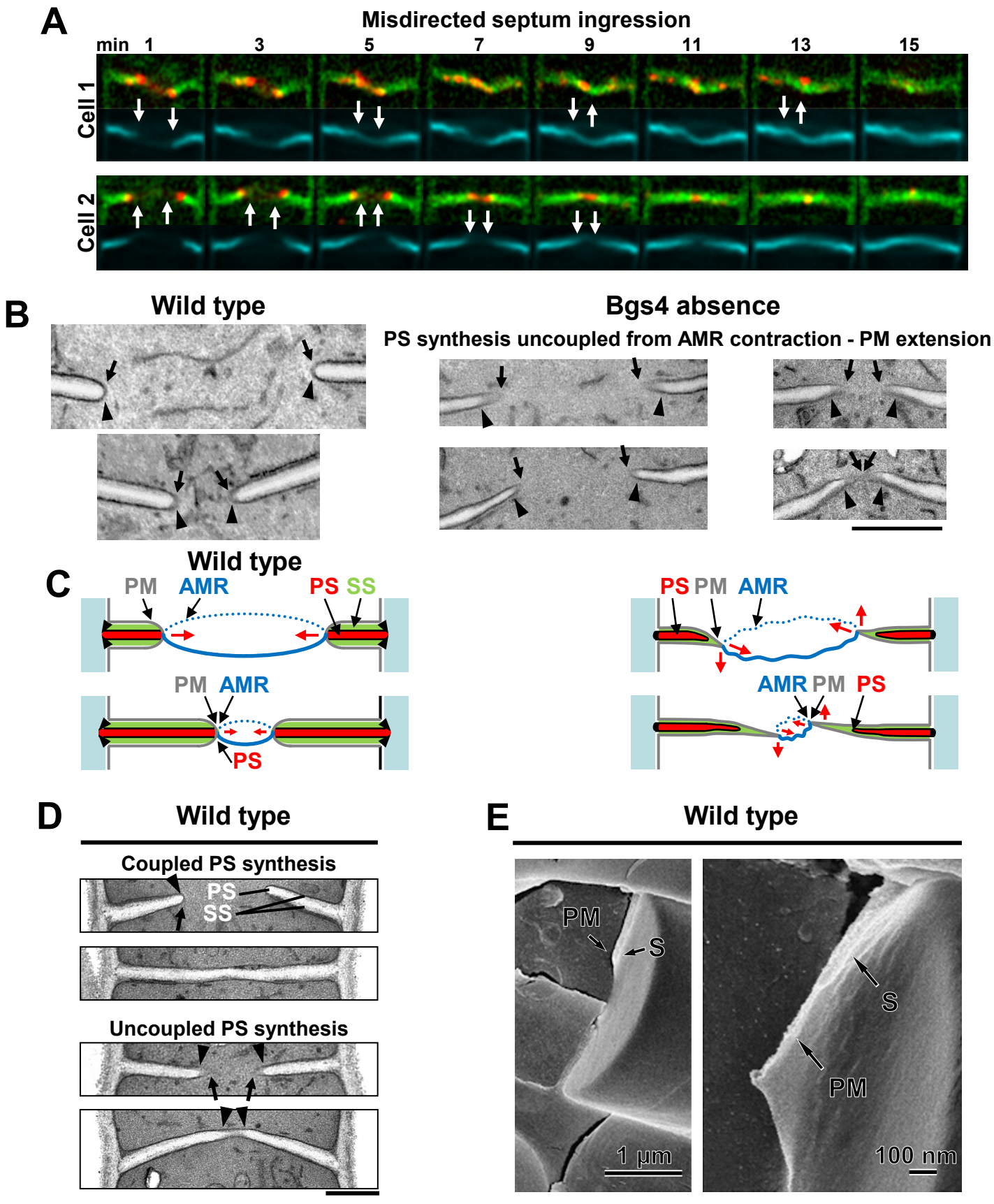

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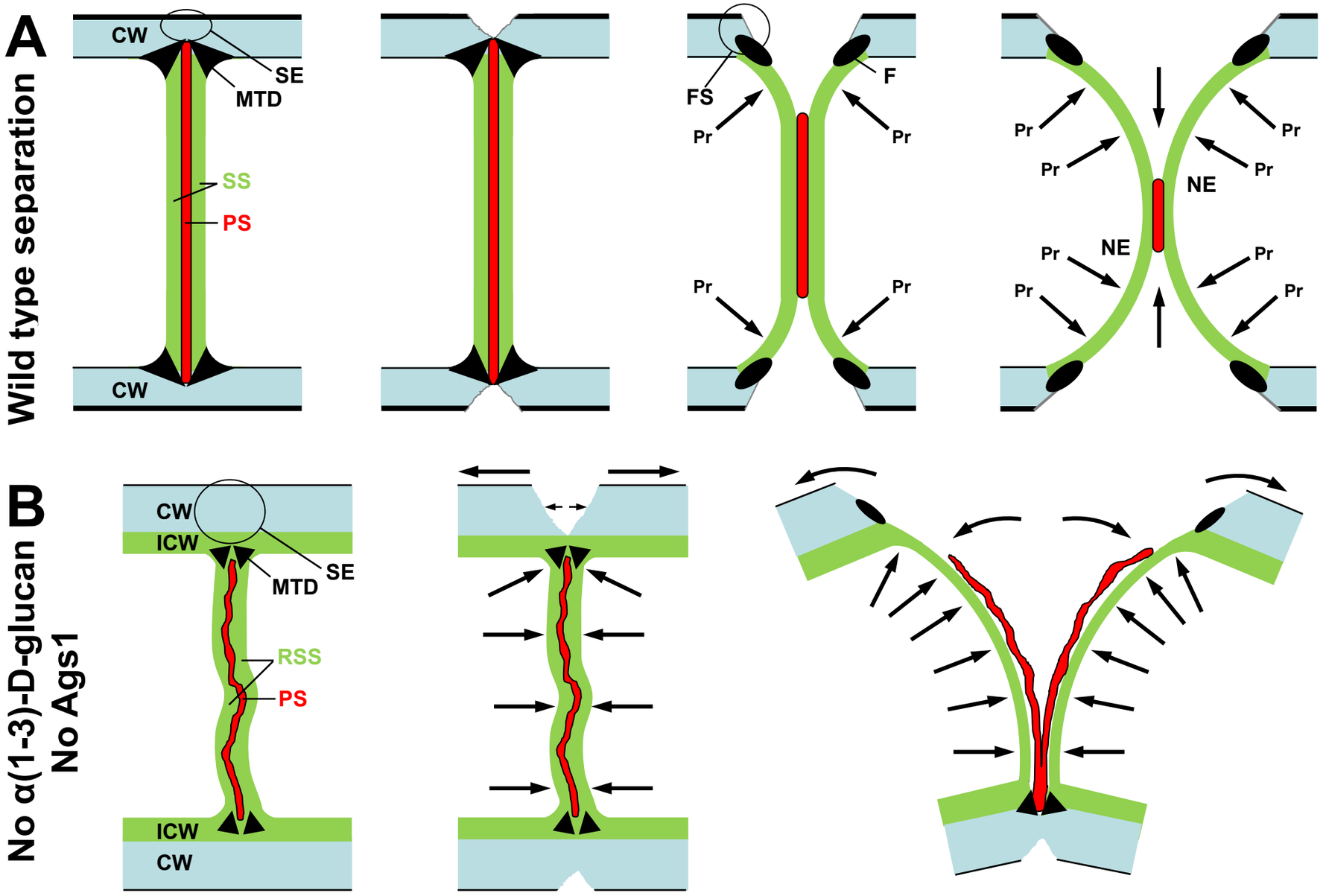

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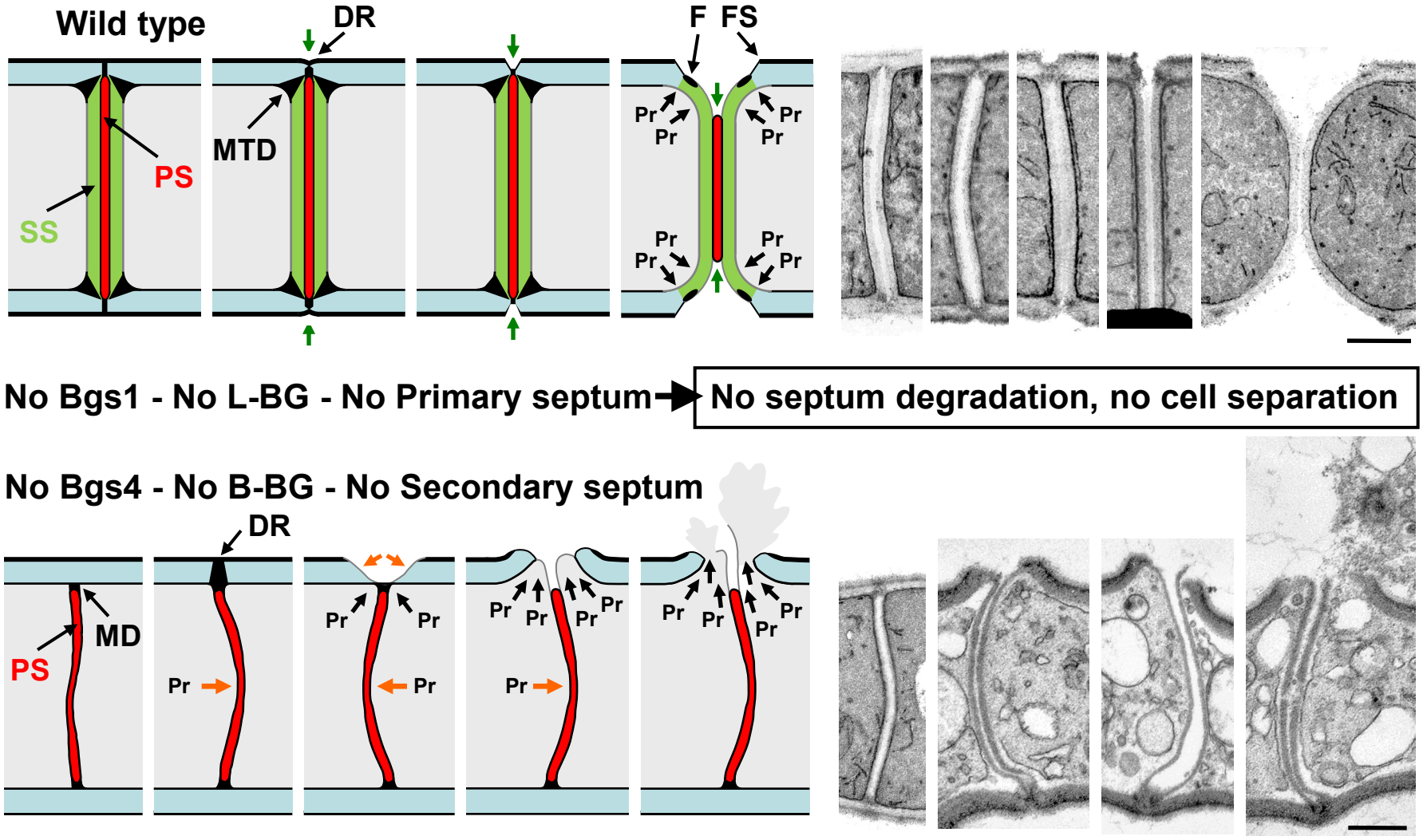

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