

1 **Overview of Fission Yeast Septation**

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1 **ABSTRACT**

2 Cytokinesis is the final process of the vegetative cycle, which divides a cell into two
3 independent daughter cells once mitosis is completed. In fungi, as in animal cells,
4 cytokinesis requires the formation of a cleavage furrow originated by constriction of an
5 actomyosin ring which is connected to the plasma membrane and causes its
6 invagination. Additionally, since fungal cells have a polysaccharide cell wall outside the
7 plasma membrane, cytokinesis requires the formation of a septum coincident with the
8 membrane ingression. Fission yeast *Schizosaccharomyces pombe* is a unicellular, rod-
9 shaped fungus that has become a popular model organism for the study of actomyosin
10 ring formation and constriction during cell division. Here we review the current
11 knowledge of the septation and separation processes in this fungus, as well as recent
12 advances in understanding the functional interaction between the transmembrane
13 enzymes that build the septum and the actomyosin ring proteins.

14

1 INTRODUCTION

2 Cytokinesis is the final stage of the eukaryotic cell cycle during which, after mitotic
3 exit, the formation of a cleavage furrow separates the cell giving rise to two new cells.
4 Cell division in fungal and animal cells is well conserved. Cleavage furrow formation
5 always requires the establishment and closure of a cytokinetic actomyosin ring (AR). A
6 major difference between fungal and animal cells is that fungi are surrounded by a rigid
7 cell wall; therefore, in fungal cells AR contraction occurs simultaneously with the
8 biosynthesis of a cell wall structure known as a septum (Willet *et al.*, 2015b; Rincón
9 and Paoletti, 2016) (**Figure 1A**). In unicellular fungi such as yeasts, at the end of
10 cytokinesis there is a controlled septum degradation that separates the two daughter
11 cells. The fission yeast *Schizosaccharomyces pombe* has been used as a model organism
12 to study the eukaryotic cytokinesis because of the high degree of conservation among
13 AR components throughout evolution. *S. pombe* is a simple, genetically tractable
14 organism with highly regular rod-shaped, stable growth patterns (Mitchison *et al.*,
15 1985). Additionally, *S. pombe* divides symmetrically, giving rise to two daughter cells of
16 the same size (Mitchison, 1957). In contrast, *Saccharomyces cerevisiae* and other yeasts
17 grow asymmetrically forming a bud that will give rise to a daughter cell. They also
18 divide asymmetrically, with the mother cell larger than the daughter cell. Therefore, the
19 positioning of the division plane in the geometrical center of *S. pombe* cells is different
20 from *S. cerevisiae* and similar to the majority of animal cells (Balasubramanian *et al.*,
21 2004). Several recent reviews discuss the regulatory mechanisms that control division
22 plane positioning in *S. pombe*, which proceeds through the assembly of cytokinetic
23 precursors on the medial cortex into nodes that coalesce into AR (Pollard and Wu, 2010;
24 Lee *et al.*, 2012; Willet *et al.*, 2015b; Rincón and Paoletti, 2016). Here we review the
25 current knowledge of septation in the fission yeast, emphasizing the importance of

1 correct septum formation for cell integrity and survival especially during cell separation.
2 Additionally, we discuss recent advances on the cooperation between the AR and
3 septum during the cleavage furrow ingression.

4

5 **Cell wall and septum composition in fission yeast**

6 All fungi contain a polysaccharide cell wall which includes glucans and mannoproteins
7 as major components. Two types of glucans are the major structural components of
8 fission yeast cell wall: branched $\beta(1,3)$ -D-glucan with 14% of $\beta(1,6)$ branches
9 constitutes 48-54% of total cell wall polysaccharides, and $\alpha(1,3)$ -D-glucan which
10 constitutes 28-32% (reviewed in Durán *et al.*, 2004). Additionally, there is a small
11 amount of linear $\beta(1,3)$ -D-glucan, mainly present in the primary septum and cell tips
12 (Cortés *et al.*, 2007). This glucan might play a role similar to chitin, present in most
13 fungi but not found in *S. pombe* (Kreger, 1954; Horisberger *et al.*, 1978). There is also a
14 small proportion of $\beta(1,6)$ -D-glucan that might be important for cross-linking different
15 polysaccharides (Magnelli *et al.*, 2005), and of galactomannan linked to the cell wall
16 glycoproteins (Ballou *et al.*, 1994).

17 Analysis by transmission electron microscopy (TEM) has found the septum to be a
18 three-layered structure with a central primary septum (PS) flanked by two layers of
19 secondary septum (SS) (Johnson *et al.*, 1973) (**Figure 1B**). The SS deposition is
20 simultaneous to the PS growth (Cortés *et al.*, 2007). The PS is a special layer of the cell
21 wall that in fission yeast is rich in linear $\beta(1,3)$ -D-glucan. In *S. cerevisiae* and other
22 fungi the PS is mainly made of chitin (Cabib *et al.*, 2005); the SS contains the same
23 polymers of the cell wall (Humbel *et al.*, 2001).

1 Fungal wall $\beta(1,3)$ -D-glucan is synthesized by the enzymatic complex $\beta(1,3)$ -D-glucan
2 synthase (EC 2.4.1.34, UDP-glucose:1,3- β -D-glucan 3- β -D-glucosyltransferase). This
3 complex, conserved in all fungi, includes at least two proteins: a catalytic subunit that is
4 a large protein with several transmembrane domains, and the Rho1 GTPase acting as
5 regulatory subunit that activates the catalytic subunit when bound to GTP (Arellano *et*
6 *al.*, 1996; Drgonova *et al.*, 1996). Different paralog genes coding for the catalytic
7 subunit, named Fks or Bgs, are present in fungal cells (Free, 2013). In budding yeast
8 and other fungi, these subunits have partially redundant roles (Mazur *et al.*, 1995) while
9 each of the four subunits present in fission yeast is essential. Bgs1, 3, and 4 function
10 during vegetative growth and Bgs2 functions during sporulation. Bgs1,3 and 4 localize
11 to growing poles, division area, and sites of wall synthesis during sexual differentiation
12 (Roncero *et al.*, 2010). Ultrastructural analysis of the septa formed in *bgs1* Δ
13 germinating spores established that Bgs1 is responsible for the linear $\beta(1,3)$ -D-glucan
14 synthesis and PS formation (Cortés *et al.*, 2007) (**Figure 1B**). The function of Bgs3 is
15 not yet known. Bgs4 is responsible for the synthesis of the major cell wall β -glucan, and
16 is essential for the maintenance of cell integrity especially during cell separation, SS
17 formation, and for correct PS completion (Cortés *et al.*, 2005; Muñoz *et al.*, 2013)
18 (**Figure 1B**).

19 The only enzyme identified as a putative α -glucan synthase is Ags1, also named Mok1.
20 Like the Bgs enzymes, Ags1 is a membrane protein essential for cell integrity and is
21 detected at the growing poles and the septum (Katayama *et al.*, 1999). Ags1 is required
22 for SS formation (**Figure 1B**) and for gradual and balanced cell separation (Cortés *et al.*
23 2012). Ags1 orthologs are not found in budding yeasts but are widely extended in other
24 fungi although they are not always essential (Edwards *et al.*, 2011; Henry *et al.*, 2012).

25

1 **Establishment of the septum position**

2 The position of the septum depends on the AR formed at the middle of the cell cortex.
3 In fission yeast, the nucleus and the anillin Mid1 mark the position of AR assembly
4 which is initiated by the maturation of medial cortical nodes (reviewed in Willet *et al.*,
5 2015b; Rincón and Paoletti, 2016). Once the ring is formed, it is necessary to keep it in
6 position until constriction starts (Wu *et al.*, 2003; Arasada and Pollard 2014; McDonald
7 *et al.*, 2016). In spherical protoplasts deprived of the wall, the AR slides to the poles
8 (Mishra *et al.*, 2012) suggesting that the AR needs to be anchored through the
9 membrane to the extracellular cell wall, and that the cylindrical shape of fission yeasts
10 might also play a role in the AR stability (Mishra *et al.*, 2012). However, it has been
11 shown in cylindrical cells that the β -glucan synthesized by Bgs4 plays a main role in
12 maintaining the AR in the cell middle before septum formation begins (Muñoz *et al.*,
13 2013). Bgs1 has also been implicated in the maintaining of AR position. Accordingly,
14 the AR of cells carrying *cps1-191*, a temperature-sensitive allele of *bgs1*⁺, slides along
15 the plasma membrane (Arasada *et al.*, 2014).

16

17 **Coupling AR contraction with septum synthesis.**

18 The septation initiation network (SIN) is a kinase cascade that activates Sid2, a kinase
19 from the NDR (Nuclear Dbf-2-related) family. Sid2 is essential in the regulation of
20 cytokinesis (reviewed in Simanis, 2015). The SIN is orthologous to the mitotic exit
21 network (MEN) in budding yeast, and the Hippo pathway in animal cells (Hergovich *et*
22 *al.*, 2006). The SIN cooperates in the regulation of mitotic entry, spindle elongation and
23 checkpoint inactivation, telophase nuclear positioning, assembly of AR, and,
24 importantly, it is essential for the AR contraction and concomitant synthesis of the

1 septum (reviewed in Simanis, 2015). Additionally the SIN inhibits a second NDR
2 kinase pathway called the morphogenesis Orb6 (MOR) network (Ray *et al.*, 2010),
3 which is orthologous to the network called regulation of Ace2 and morphogenesis
4 (RAM) in *S. cerevisiae* and other fungi (Saputo *et al.*, 2012). The MOR network is
5 required for cell separation and apical growth (Gupta *et al.*, 2014). Most SIN
6 components are essential, and temperature-sensitive SIN mutants form defective AR but
7 do not initiate septum synthesis, leading to the formation of elongated multinucleated
8 cells at the restrictive temperature (reviewed in Krapp *et al.*, 2008; Simanis, 2015).
9 Because Rho1 GTPase can rescue some SIN mutants, it has been proposed that SIN
10 activates Rho1, which in turn activates the Bgs enzymes, (Jin *et al.*, 2006), but it has not
11 been proved. Additionally, it has been proposed that there is a feedback loop where Rho1
12 activates the SIN to ensure SIN activity while septation is progressing (Alcaide-Gavilan
13 *et al.*, 2014).

14 In animal cells AR constriction is dependent on myosin type II and exerts the force
15 needed to pull the plasma membrane and form the cleavage furrow. In fungi AR
16 constriction is also myosin II dependent (Mishra *et al.*, 2013) and is required for the
17 initiation of septum formation, but does not provide the pulling force for the furrow
18 ingression (Proctor *et al.*, 2012). Since AR constriction and septum synthesis occur
19 simultaneously (**Figure 1A**), it has been proposed that linear $\beta(1,3)$ -D-glucan synthesis
20 provides the major force for the furrow closure (Proctor *et al.*, 2012). On the other hand,
21 the AR is dispensable when the septum is already forming but ingression is much
22 slower in its absence (Proctor *et al.*, 2012). It seems therefore that ring constriction
23 activates septum synthesis. Supporting this hypothesis, two recent works propose that
24 septum synthesis is mechanosensitive and somehow coupled to contractile AR tension
25 (Thiyagarajan *et al.*, 2015; Zhou *et al.*, 2015). By manipulating the curvature of the

1 cleavage furrow it was shown that the AR promotes local septum growth in a curvature-
2 dependent manner suggesting that Bgs1 is regulated by AR tension (Zhou *et al.*, 2015).
3 Conversely, as mentioned above, when Bgs1 is defective the AR slides (Arasada *et al.*,
4 2014), is disorganized (Cortés *et al.*, 2015), and does not contract (Liu *et al.*, 1999).
5 Whether and how the AR contractile force stimulates the cell wall machinery, and how
6 the cell wall maintains the AR and stimulates its contraction are currently major
7 questions in fungal septation.

8 Focal adhesions in animal cells connect the extracellular matrix to the cytoskeleton and
9 transmits signals in both directions. Similarly, during fission yeast cytokinesis several
10 proteins might form a complex to connect the cell wall with the AR through the plasma
11 membrane. Major candidates to organize this complex are the F-BAR proteins (Roberts-
12 Galbraith and Gould, 2010), which can bridge the plasma membrane and the
13 cytoskeleton. Cdc15 is the founding member of the PCH family (Carnahan and Gould,
14 2003; Wu *et al.*, 2003) and contributes to AR formation via the direct binding of its F-
15 BAR domain to the formin Cdc12 that nucleates the AR actin filaments (Willet *et al.*,
16 2015a). Additionally, Cdc15 contains an SH3 domain that binds proteins required for
17 septation such as Px11, Fic1, or Rgf3 (Roberts-Galbraith *et al.*, 2009; Ren *et al.*, 2015).
18 A second F-BAR protein named Imp2 (Demeter *et al.*, 1998) also has an SH3 domain
19 functionally interchangeable with that of Cdc15 and both collaborate in the recruitment
20 of the mentioned proteins (Roberts-Galbraith *et al.*, 2009; Ren *et al.*, 2015). It has been
21 recently described that Cdc15 oligomerization is critical for fission yeast cytokinesis
22 (McDonald *et al.*, 2015). Px11 is the fission yeast ortholog of animal cells paxillin, it
23 binds to myosin II and stabilizes the AR (Ge *et al.*, 2008; Pinar *et al.*, 2008). Px11
24 collaborates with Bgs1 to maintain the AR and form the septum (Cortés *et al.*, 2015).
25 Bgs1 depletion generates abnormal septa made of SS layers, which somehow are guided

1 by the AR (**Figure 1B**). When Bgs1 depletion occurs in the absence of Pxl1 there is no
2 septum synthesis initiation (**Figure 1B**). It is possible that Pxl1, by binding to myosin II
3 and Cdc15 (Cortés *et al.*, 2015; Ren *et al.*, 2015), transmits the AR tension to the
4 membrane in order to concentrate the Ags1 and Bgs4 synthases and narrow the area of
5 septum synthesis (**Figure 2**). Fic1 is a C2-domain containing protein whose role is not
6 defined in *S. pombe* but which becomes essential in the absence of the Pxl1 (Roberts-
7 Galbraith *et al.*, 2009, Ren *et al.*, 2015). Rgf3 is an essential GEF that activates Rho1
8 (Arellano *et al.*, 1996; Tajadura *et al.*, 2004; Morrell-Falvey *et al.*, 2005; Mutoh *et al.*,
9 2005). In turn, this GTPase directly activates the Bgs enzymes and glucan synthesis
10 (Arellano *et al.*, 1996; Tajadura *et al.*, 2004; Morrell-Falvey *et al.*, 2005; Mutoh *et al.*,
11 2005). Whether or how Rgf3 and Pxl1 functionally interact to transform the AR
12 contraction into an activation signal for the biosynthetic enzymes that form the septum
13 remains to be discovered.

14 Another F-BAR protein, Rga7, forms a complex with Cdc15 and Imp2 (Martín-García
15 *et al.*, 2014). Rga7 also contains a Rho GTPase activating protein (GAP) domain and
16 acts as negative regulator of Rho2 (Martin-García *et al.*, 2014). These complex
17 networks of proteins might contribute to the coordination of contractile ring constriction
18 and septum formation (**Figure 2**).

19 F-BAR proteins may also have a role in the traffic of Bgs enzymes. Two recent works
20 propose that Cdc15 participates in the transport of Bgs1 from late Golgi to the
21 membrane at the division area, and similarly, Rga7 contributes to the transfer of the
22 Bgs4 (Arasada *et al.*, 2014, Arasada *et al.*, 2015). The role of exocytosis, endocytosis,
23 and membrane traffic during the formation of the septum is only beginning to be
24 uncovered.

1

2 **Cell separation and cell integrity**

3 Separation is the most critical process of the cell cycle for the cell integrity. In animal
4 cells, the terminal step of cytokinesis is called abscission and includes microtubule
5 severing and membrane splitting that is mediated by the endosomal sorting complex
6 required for transport (ESCRT) proteins (Bhutta *et al.*, 2014). In filamentous fungi cell
7 separation does not occur and they form hyphae composed of cell compartments
8 delimited by septa with a small central pore. These septa are important for maintaining
9 hyphal integrity. Thus, they are sealed immediately upon injury impeding an extensive
10 loss of cytoplasm (Mourino-Perez *et al.*, 2013).

11 Cell separation does not seem to be essential for the vegetative cycle of the fission
12 yeast. A broad range of viable mutants defective in cell separation have been described
13 in the past (reviewed in Sipiczki, 2007), and recently a systematic visual screening of
14 the deletion collection of *S. pombe* haploids identified new genes based on the “long
15 branched” phenotype of viable cells (Hayles *et al.*, 2013). Not all of these genes
16 participate directly in the process of cell separation but play a role in earlier steps of
17 cytokinesis. Thus, most cells lacking Pxl1, or temperature-sensitive SIN mutants grown
18 at semi-restrictive temperature are septated even if they do not have a separation defect.
19 It is possible that the separation machinery, which at least includes the septins, the
20 exocyst, Rho GTPases, and glucanases (reviewed in Martín-García and Santos 2016), is
21 set at the beginning of septation, and if the AR constriction/septation is delayed,
22 separation can no longer take place. The Sep1-Ace2 transcription-factor cascade
23 regulates the periodic expression of many genes encoding proteins required for AR
24 constriction and for cell separation (Rustici *et al.*, 2004; Alonso-Nuñez *et al.*, 2005).

1 Additionally, a posttranslational control on cell separation might be exerted during
2 cytokinesis by the SIN, which inhibits the MOR pathway (Gupta *et al.*, 2014). This
3 pathway regulates septum degradation although the mechanism is not yet known (Gupta
4 *et al.*, 2014).

5 To prevent cell lysis during separation, a precisely controlled degradation of the lateral
6 cell wall at the division area and the PS is required. The remaining SS gradually curves
7 concavely due to the internal cell pressure, and forms the new end in the daughter cells.
8 (Cortés *et al.*, 2012; Atilgan *et al.*, 2015). Bgs4 depletion induces unopposed cell wall
9 degradation leaving the plasma membrane without cell wall and consequently, the
10 internal turgor pressure causes the cell lysis (Cortés *et al.*, 2005; Muñoz *et al.*, 2013). In
11 Ags1-depleted cells lysis also occurs during separation suggesting that a correct SS
12 assembly is essential for cell viability (Cortés *et al.*, 2012).

13 Degradation of the lateral cell wall requires the Agn1 1,3- α -glucanase (Dekker *et al.*,
14 2004; Garcia *et al.*, 2005), and Eng1 1,3- β -glucanase is necessary to digest the PS
15 (Alonso-Nuñez *et al.*, 2005). Precise secretion of these enzymes involves the formation
16 of septin rings and the directed activity of the exocyst complex (Martin-Cuadrado *et al.*,
17 2005). Rho4 GTPase is also required for glucanase secretion (Santos *et al.*, 2005), likely
18 through exocyst regulation (Perez *et al.*, 2015). This GTPase is activated by Gef3,
19 which interacts with and is localized by the septins (Muñoz *et al.*, 2014; Wang *et al.*,
20 2015). In this way, a precise spatio-temporal regulation of glucanase secretion preserves
21 cell integrity.

22

23 **Concluding remarks**

1 Although this review focuses on fission yeast, similar mechanisms of septation exist in
2 other fungi. There are still a number of open questions on the septation process that
3 need to be further addressed: the targets of the SIN that activate septation; the different
4 functions of F-BAR proteins during septum formation; the role of Rho GTPases and
5 other molecules that regulate cell wall synthesis; etc. In all these questions the
6 connection between the cell wall and the AR through the plasma membrane is emerging
7 as an important condition for a successful cytokinesis and for the maintenance of cell
8 integrity. Although it is still unknown how this connection is accomplished, some of the
9 main players such as Bgs enzymes, F-BAR proteins, Pxl1, and other cytoskeleton
10 binding proteins have been already identified. The characterization of new double
11 conditional mutants, proteomics, and high-resolution microscopy techniques will help to
12 further characterize this connection.

13

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20 **FIGURE LEGENDS**

21 **Figure 1.** A) Time-lapse fluorescence micrographs showing simultaneously AR
22 constriction and septum synthesis by using cells expressing GFP-cdc15 to label the AR,
23 RFP-Bgs1 to label the membrane and calcofluor (CW) staining to label the cell wall

1 glucan. Bar 5 μm . B) The ultrastructure of the *Schizosaccharomyces pombe* septum.
2 Transmission electron micrographs of wild type septum showing the middle layer of PS
3 flanked by the SS; septum of cells with repressed *bgs4*⁺ showing no SS and floppy PS
4 (adapted from Muñoz *et al.*, 2013); septum of cells with repressed *ags1*⁺ showing
5 defective SS and floppy PS (adapted from Cortés *et al.*, 2012); with repressed *bgs1*⁺
6 showing parallel SS depositions in wild type cells and absence of septum in cells
7 lacking Pxl1 (adapted from Cortés *et al.*, 2015). Bar 1 μm .

8 **Figure 2.** Model of the protein complexes connecting AR, septum membrane, and
9 septum wall.

10 **Figure 3.** A) Model of cell separation at the end of cytokinesis. B, C) Transmission
11 electron micrograph showing how degradation of the lateral cell wall (B) and PS (C)
12 leaves the SS that, upon separation, changes from flat to round shape (adapted from
13 Cortés *et al.*, 2012).

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18 **BIBLIOGRAPHY**

- 19 Alcaide-Gavilan, M., Lahoz, A., Daga, R.R. and Jimenez, J. (2014). Feedback
20 regulation of SIN by Etd1 and Rho1 in fission yeast. *Genetics* **196**, 455-470.
21 Alonso-Nuñez, M.L., An, H., Martín-Cuadrado, A.B., Mehta, S., Petit, C., Sipiczki, M.,
22 *et al.* (2005). Ace2p controls the expression of genes required for cell separation
23 in *Schizosaccharomyces pombe*. *Mol Biol Cell* **16**, 2003-2017.
24 Arasada, R. and Pollard, T.D. (2014). Contractile Ring Stability in *S. pombe* Depends on
25 F-BAR Protein Cdc15p and Bgs1p Transport from the Golgi Complex. *Cell Rep*
26 **8**, 1533-1544.

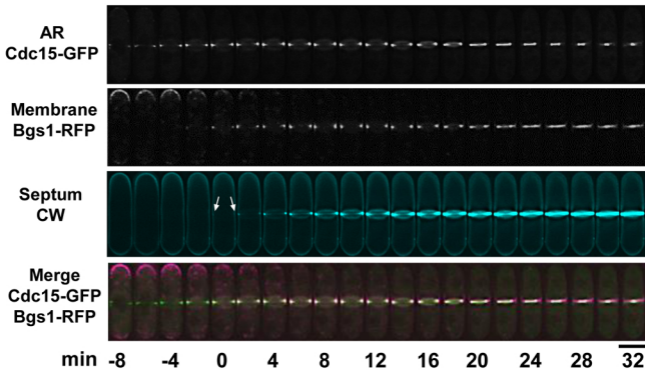
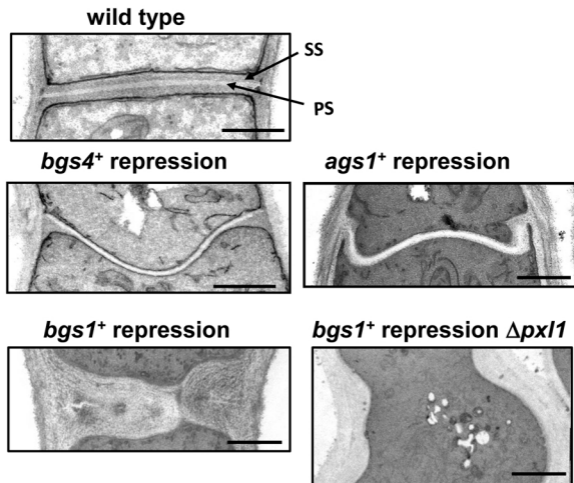
- 1 Arasada, R. and Pollard, T.D. (2015). A role for F-BAR protein Rga7p during
2 cytokinesis in *S. pombe*. *J Cell Sci* **128**, 2259-2268.
- 3 Arellano, M., Durán, A. and Pérez, P. (1996). Rho 1 GTPase activates the (1-3)beta-D-
4 glucan synthase and is involved in *Schizosaccharomyces pombe* morphogenesis.
5 *EMBO J* **15**, 4584-4591.
- 6 Atilgan, E., Magidson, V., Khodjakov, A. and Chang, F. (2015). Morphogenesis of the
7 Fission Yeast Cell through Cell Wall Expansion. *Curr Biol* **25**, 2150-2157.
- 8 Balasubramanian, M.K., Bi, E. and Glotzer, M. (2004). Comparative analysis of
9 cytokinesis in budding yeast, fission yeast and animal cells. *Curr Biol* **14**, R806-
10 818.
- 11 Bhutta, M.S., McInerney, C.J. and Gould, G.W. (2014). ESCRT function in cytokinesis:
12 location, dynamics and regulation by mitotic kinases. *Int J Mol Sci* **15**, 21723-
13 21739.
- 14 Ballou, C.E., Ballou, L., and Ball, G. (1994). *Schizosaccharomyces pombe*
15 glycosylation mutant with altered cell surface properties. *Proc Natl Acad Sci U S*
16 *A* **91**, 9327-9331.
- 17 Cabib, E. and Durán, A. (2005). Synthase III-dependent chitin is bound to different
18 acceptors depending on location on the cell wall of budding yeast. *J Biol Chem*
19 **280**, 9170-9179.
- 20 Carnahan, R.H., and Gould, K.L. (2003). The PCH family protein, Cdc15p, recruits two
21 F-actin nucleation pathways to coordinate cytokinetic actin ring formation in
22 *Schizosaccharomyces pombe*. *J Cell Biol* **162**, 851-862.
- 23 Cortés, J.C.G., Carnero, E., Ishiguro, J., Sanchez, Y., Durán, A. and Ribas, J.C. (2005).
24 The novel fission yeast (1,3)b-D-glucan synthase catalytic subunit Bgs4p is
25 essential during both cytokinesis and polarized growth. *J Cell Sci* **118**, 157-174.
- 26 Cortés, J.C.G., Konomi, M., Martins, I.M., Muñoz, J., Moreno, M.B., Osumi, M., *et al.*
27 (2007). The (1,3)b-D-glucan synthase subunit Bgs1p is responsible for the
28 fission yeast primary septum formation. *Mol Microbiol* **65**, 201-217.
- 29 Cortés, J.C.G., Pujol, N., Sato, M., Pinar, M., Ramos, M., Moreno, B., *et al.* (2015).
30 Cooperation between Paxillin-like Protein Pxl1 and Glucan Synthase Bgs1 Is
31 Essential for Actomyosin Ring Stability and Septum Formation in Fission Yeast.
32 *PLoS Genet* **11**, e1005358.
- 33 Cortés, J.C.G., Sato, M., Muñoz, J., Moreno, M.B., Clemente-Ramos, J.A., Ramos, M.,
34 *et al.* (2012). Fission yeast Ags1 confers the essential septum strength needed for
35 safe gradual cell abscission. *J Cell Biol* **198**, 637-656.
- 36 Dekker, N., Speijer, D., Grun, C.H., van den Berg, M., de Haan, A. and Hochstenbach,
37 F. (2004). Role of the alpha-glucanase Agn1p in fission-yeast cell separation.
38 *Mol Biol Cell* **15**, 3903-3914.
- 39 Demeter, J. and Sazer, S. (1998). imp2, a new component of the actin ring in the fission
40 yeast *Schizosaccharomyces pombe*. *J Cell Biol* **143**, 415-427.
- 41 Drgonova, J., Drgon, T., Tanaka, K., Kollar, R., Chen, G.C., Ford, R.A., *et al.* (1996).
42 Rho1p, a yeast protein at the interface between cell polarization and
43 morphogenesis. *Science* **272**, 277-279.
- 44 Durán, A. and Pérez, P. (2004) Cell Wall Synthesis. In *The Molecular Biology of*
45 *Schizosaccharomyces pombe*, R. Egel (ed.). Springer-Verlag Berlin Heidelberg,
46 pp. 269-279.
- 47 Edwards, J.A., Alore, E.A. and Rappleye, C.A. (2011). The yeast-phase virulence
48 requirement for alpha-glucan synthase differs among *Histoplasma capsulatum*
49 chemotypes. *Eukaryot Cell* **10**, 87-97.
- 50 Free, S.J. (2013). Fungal cell wall organization and biosynthesis. *Adv Genet* **81**, 33-82.

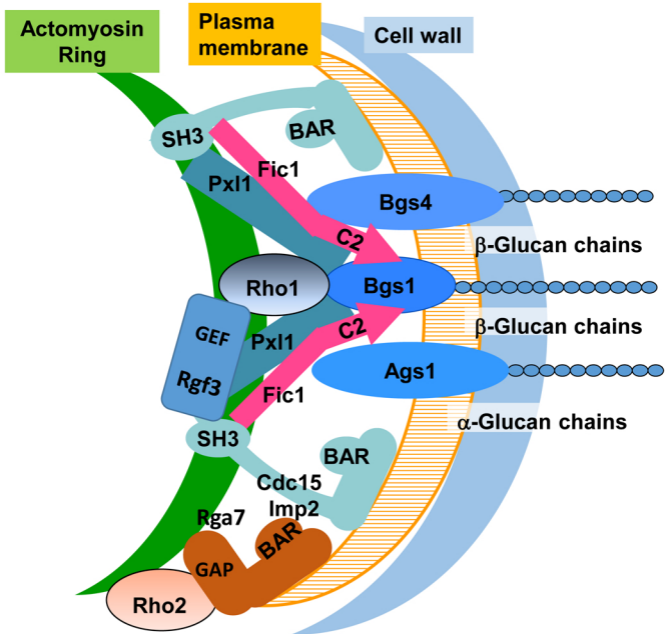
- 1 Garcia, I., Jimenez, D., Martin, V., Duran, A. and Sanchez, Y. (2005). The alpha-
2 glucanase Agn1p is required for cell separation in *Schizosaccharomyces pombe*.
3 *Biol Cell* **97**, 569-576.
- 4 Ge, W. and Balasubramanian, M.K. (2008). Pxl1p, a paxillin-related protein, stabilizes
5 the actomyosin ring during cytokinesis in fission yeast. *Mol Biol Cell* **19**, 1680-
6 1692.
- 7 Gupta, S., Govindaraghavan, M. and McCollum, D. (2014). Cross talk between NDR
8 kinase pathways coordinates cytokinesis with cell separation in
9 *Schizosaccharomyces pombe*. *Eukaryot Cell* **13**, 1104-1112.
- 10 Hayles, J., Wood, V., Jeffery, L., Hoe, K.L., Kim, D.U., Park, H.O., *et al.* (2013). A
11 genome-wide resource of cell cycle and cell shape genes of fission yeast. *Open*
12 *Biol* **3**, 130053.
- 13 Henry, C., Latge, J.P. and Beauvais, A. (2012). alpha1,3 glucans are dispensable in
14 *Aspergillus fumigatus*. *Eukaryot Cell* **11**, 26-29.
- 15 Hergovich, A., Stegert, M.R., Schmitz, D. and Hemmings, B.A. (2006). NDR kinases
16 regulate essential cell processes from yeast to humans. *Nat Rev Mol Cell Biol* **7**,
17 253-264.
- 18 Horisberger, M., Vonlanthen, M. and Rosset, J. (1978). Localization of alpha-
19 galactomannan and of wheat germ agglutinin receptors in *Schizosaccharomyces*
20 *pombe*. *Arch Microbiol* **119**, 107-111.
- 21 Humbel, B.M., Konomi, M., Takagi, T., Kamasawa, N., Ishijima, S.A. and Osumi, M.
22 (2001). In situ localization of beta-glucans in the cell wall of
23 *Schizosaccharomyces pombe*. *Yeast* **18**, 433-444.
- 24 Jin, Q.W., Zhou, M., Bimbo, A., Balasubramanian, M.K. and McCollum, D. (2006). A
25 role for the septation initiation network in septum assembly revealed by genetic
26 analysis of sid2-250 suppressors. *Genetics* **172**, 2101-2112.
- 27 Johnson, B.F., B.Y. Yoo, and G.B. Calleja. 1973. Cell division in yeasts: movement of
28 organelles associated with cell plate growth of *Schizosaccharomyces pombe*. *J.*
29 *Bacteriol.* **115**:358–366.
- 30 Katayama, S., Hirata, D., Arellano, M., Pérez, P. and Toda, T. (1999). Fission yeast a-
31 glucan synthase Mok1 requires the actin cytoskeleton to localize the sites of
32 growth and plays an essential role in cell morphogenesis downstream of protein
33 kinase C function. *J Cell Biol* **144**, 1173-1186.
- 34 Krapp, A. and Simanis, V. (2008). An overview of the fission yeast septation initiation
35 network (SIN). *Biochem Soc Trans* **36**, 411-415.
- 36 Kreger, D.R. (1954). Observations on cell walls of yeasts and some other fungi by x-ray
37 diffraction and solubility tests. *Biochim Biophys Acta* **13**, 1-9.
- 38 Lee, I.J., Coffman, V.C. and Wu, J.Q. (2012). Contractile-ring assembly in fission yeast
39 cytokinesis: Recent advances and new perspectives. *Cytoskeleton (Hoboken)* **69**,
40 751-763.
- 41 Liu, J., Wang, H., McCollum, D. and Balasubramanian, M.K. (1999). Drc1p/Cps1p, a
42 1,3-beta-glucan synthase subunit, is essential for division septum assembly in
43 *Schizosaccharomyces pombe*. *Genetics* **153**, 1193-1203.
- 44 Magnelli, P.E., Cipollo, J.F. and Robbins, P.W. (2005). A glucanase-driven fractionation
45 allows redefinition of *Schizosaccharomyces pombe* cell wall composition and
46 structure: assignment of diglucan. *Anal Biochem* **336**, 202-212.
- 47 Martin-Cuadrado, A.B., Morrell, J.L., Konomi, M., An, H., Petit, C., Osumi, M., *et al.*
48 (2005). Role of septins and the exocyst complex in the function of hydrolytic
49 enzymes responsible for fission yeast cell separation. *Mol Biol Cell* **16**, 4867-
50 4881.

- 1 Martin-Garcia, R., Coll, P.M. and Perez, P. (2014). F-BAR domain protein Rga7
2 collaborates with Cdc15 and Imp2 to ensure proper cytokinesis in fission yeast.
3 *J Cell Sci* **127**, 4146-4158.
- 4 Martin-Garcia, R., and Santos, B. (2016). The price of independence: cell separation in
5 fission yeast. *World J Microbiol Biotechnol* **32**, 65.
- 6 McDonald, N.A., Vander Kooi, C.W., Ohi, M.D., and Gould, K.L. (2015).
7 Oligomerization but Not Membrane Bending Underlies the Function of Certain
8 F-BAR Proteins in Cell Motility and Cytokinesis. *Dev Cell* **35**, 725-736.
- 9 McDonald, N.A., Takizawa, Y., Feoktistova, A., Xu, P., Ohi, M.D., Vander Kooi, C.W.
10 and Gould, K.L. (2016). The Tubulation Activity of a Fission Yeast F-BAR
11 Protein Is Dispensable for Its Function in Cytokinesis. *Cell Rep* **14**, 534-546.
- 12 Mishra, M., Huang, Y., Srivastava, P., Srinivasan, R., Sevugan, M., Shlomovitz, R., *et*
13 *al.* (2012). Cylindrical cellular geometry ensures fidelity of division site
14 placement in fission yeast. *J Cell Sci* **125**, 3850-3857.
- 15 Mishra, M., Kashiwazaki, J., Takagi, T., Srinivasan, R., Huang, Y., Balasubramanian,
16 M.K. and Mabuchi, I. (2013). In vitro contraction of cytokinetic ring depends on
17 myosin II but not on actin dynamics. *Nat Cell Biol* **15**, 853-859.
- 18 Mitchison, J.M. (1957). The growth of single cells. I. *Schizosaccharomyces pombe*. *Exp*
19 *Cell Res* **13**, 244-262.
- 20 Mitchison, J.M. and Nurse, P. (1985). Growth in cell length in the fission yeast
21 *Schizosaccharomyces pombe*. *J Cell Sci* **75**, 357-376.
- 22 Morrell-Falvey, J.L., Ren, L., Feoktistova, A., Haese, G.D. and Gould, K.L. (2005). Cell
23 wall remodeling at the fission yeast cell division site requires the Rho-GEF
24 Rgf3p. *J Cell Sci* **118**, 5563-5573.
- 25 Mourino-Perez, R.R. and Riquelme, M. (2013). Recent advances in septum biogenesis
26 in *Neurospora crassa*. *Adv Genet* **83**, 99-134.
- 27 Muñoz, S., Manjon, E. and Sanchez, Y. (2014). The putative exchange factor Gef3p
28 interacts with Rho3p GTPase and the septin ring during cytokinesis in fission
29 yeast. *J Biol Chem* **289**, 21995-22007.
- 30 Muñoz, J., Cortés, J.C.G., Sipiczki, M., Ramos, M., Clemente-Ramos, J.A., Moreno,
31 M.B., *et al.* (2013). Extracellular cell wall b(1,3)glucan is required to couple
32 septation to actomyosin ring contraction. *J Cell Biol* **203**, 265-282.
- 33 Mutoh, T., Nakano, K. and Mabuchi, I. (2005). Rho1-GEFs Rgf1 and Rgf2 are involved
34 in formation of cell wall and septum, while Rgf3 is involved in cytokinesis in
35 fission yeast. *Genes Cells* **10**, 1189-1202.
- 36 Pérez, P., Portales, E. and Santos, B. (2015). Rho4 interaction with exocyst and septins
37 regulates cell separation in fission yeast. *Microbiology* **161**, 948-959.
- 38 Pinar, M., Coll, P.M., Rincón, S.A. and Pérez, P. (2008). *Schizosaccharomyces pombe*
39 Pxl1 is a paxillin homologue that modulates Rho1 activity and participates in
40 cytokinesis. *Mol Biol Cell* **19**, 1727-1738.
- 41 Pollard, T.D., and Wu, J.Q. (2010). Understanding cytokinesis: lessons from fission
42 yeast. *Nat Rev Mol Cell Biol* **11**, 149-155.
- 43 Proctor, S.A., Minc, N., Boudaoud, A. and Chang, F. (2012). Contributions of turgor
44 pressure, the contractile ring, and septum assembly to forces in cytokinesis in
45 fission yeast. *Curr Biol* **22**, 1601-1608.
- 46 Ray, S., Kume, K., Gupta, S., Ge, W., Balasubramanian, M., Hirata, D. and McCollum,
47 D. (2010). The mitosis-to-interphase transition is coordinated by cross talk
48 between the SIN and MOR pathways in *Schizosaccharomyces pombe*. *J Cell*
49 *Biol* **190**, 793-805.

- 1 Ren, L., Willet, A.H., Roberts-Galbraith, R.H., McDonald, N.A., Feoktistova, A., Chen,
2 J.S., *et al.* (2015). The Cdc15 and Imp2 SH3 domains cooperatively scaffold a
3 network of proteins that redundantly ensure efficient cell division in fission
4 yeast. *Mol Biol Cell* **26**, 256-269.
- 5 Rincón, S.A. and Paoletti, A. (2016). Molecular control of fission yeast cytokinesis.
6 *Semin Cell Dev Biol*.
- 7 Roberts-Galbraith, R.H., Chen, J.S., Wang, J. and Gould, K.L. (2009). The SH3
8 domains of two PCH family members cooperate in assembly of the
9 *Schizosaccharomyces pombe* contractile ring. *J Cell Biol* **184**, 113-127.
- 10 Roberts-Galbraith, R.H., and Gould, K.L. (2010). Setting the F-BAR: functions and
11 regulation of the F-BAR protein family. *Cell Cycle* **9**, 4091-4097.
- 12 Roncero, C. and Sánchez, Y. (2010). Cell separation and the maintenance of cell
13 integrity during cytokinesis in yeast: the assembly of a septum. *Yeast* **27**, 521-
14 530.
- 15 Rustici, G., Mata, J., Kivinen, K., Lio, P., Penkett, C.J., Burns, G., *et al.* (2004).
16 Periodic gene expression program of the fission yeast cell cycle. *Nat Genet* **36**,
17 809-817.
- 18 Santos, B., Martin-Cuadrado, A.B., Vazquez de Aldana, C.R., del Rey, F. and Pérez, P.
19 (2005). Rho4 GTPase is involved in secretion of glucanases during fission yeast
20 cytokinesis. *Eukaryot Cell* **4**, 1639-1645.
- 21 Saputo, S., Chabrier-Rosello, Y., Luca, F.C., Kumar, A. and Krysan, D.J. (2012). The
22 RAM network in pathogenic fungi. *Eukaryot Cell* **11**, 708-717.
- 23 Simanis, V. (2015). Pombe's thirteen - control of fission yeast cell division by the
24 septation initiation network. *J Cell Sci* **128**, 1465-1474.
- 25 Sipiczki, M. (2007). Splitting of the fission yeast septum. *FEMS Yeast Res* **7**, 761-770.
- 26 Tajadura, V., García, B., García, I., García, P. and Sánchez, Y. (2004).
27 *Schizosaccharomyces pombe* Rgf3p is a specific Rho1 GEF that regulates cell
28 wall b-glucan biosynthesis through the GTPase Rho1p. *J Cell Sci* **117**, 6163-
29 6174.
- 30 Thiyagarajan, S., Munteanu, E.L., Arasada, R., Pollard, T.D. and O'Shaughnessy, B.
31 (2015). The fission yeast cytokinetic contractile ring regulates septum shape and
32 closure. *J Cell Sci*.
- 33 Wang, N., Wang, M., Zhu, Y.H., Grosel, T.W., Sun, D., Kudryashov, D.S. and Wu, J.Q.
34 (2015). The Rho-GEF Gef3 interacts with the septin complex and activates the
35 GTPase Rho4 during fission yeast cytokinesis. *Mol Biol Cell* **26**, 238-255.
- 36 Willet, A.H., McDonald, N.A., Bohnert, K.A., Baird, M.A., Allen, J.R., Davidson, M.W.
37 and Gould, K.L. (2015a). The F-BAR Cdc15 promotes contractile ring
38 formation through the direct recruitment of the formin Cdc12. *J Cell Biol* **208**,
39 391-399.
- 40 Willet, A.H., McDonald, N.A. and Gould, K.L. (2015b). Regulation of contractile ring
41 formation and septation in *Schizosaccharomyces pombe*. *Curr Opin Microbiol*
42 **28**, 46-52.
- 43 Wu, J.Q., Kuhn, J.R., Kovar, D.R. and Pollard, T.D. (2003). Spatial and temporal
44 pathway for assembly and constriction of the contractile ring in fission yeast
45 cytokinesis. *Dev Cell* **5**, 723-734.
- 46 Zhou, Z., Munteanu, E.L., He, J., Ursell, T., Bathe, M., Huang, K.C. and Chang, F.
47 (2015). The contractile ring coordinates curvature dependent septum assembly
48 during fission yeast cytokinesis. *Mol Biol Cell* **26**, 78-90.

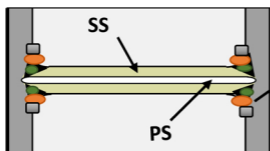
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A**B**



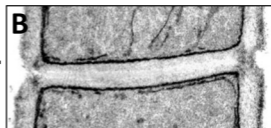
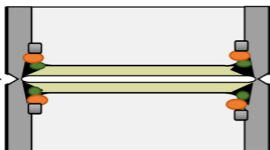
A

- Septin ring
- Exocyst complex
- Rho4 GTPase



Secretion of
Glucanases

Agn1 →



Eng1 →

