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Coordinating Septum Formation and the Actomyosin Ring during Cytokinesis in *Schizosaccharomyces pombe*.

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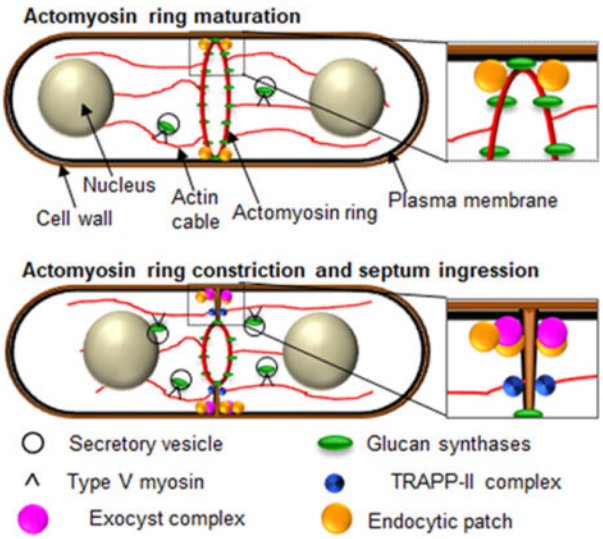
Summary

During cytokinesis, animal and fungal cells form a membrane furrow via actomyosin ring constriction. Our understanding of actomyosin ring-driven cytokinesis stems extensively from the fission yeast model system. However, unlike animal cells, actomyosin ring constriction occurs simultaneously with septum formation in fungi. While the formation of an actomyosin ring is essential for cytokinesis in fission yeast, proper furrow formation also requires septum deposition. The molecular mechanisms of spatiotemporal coordination of septum deposition with actomyosin ring constriction are poorly understood. Although, the role of the actomyosin ring as a mechanical structure driving furrow formation is better understood, its role as a spatiotemporal landmark for septum deposition is not widely discussed. Here we review and discuss recent advances describing how the actomyosin ring spatiotemporally regulates membrane traffic to promote septum driven cytokinesis in fission yeast. Finally, we explore emerging questions in cytokinesis, and discuss the role of extracellular matrix during cytokinesis in other organisms.

Graphical Abstract



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Conflict of Interest

There authors declare that they have no conflict of interests.

Cytokinesis in fission yeast involves actomyosin ring constriction that occurs simultaneously with septum (cell wall) synthesis. In this review we discuss how these two processes are coordinated and how multiple membrane trafficking events are necessary for proper cytokinesis.

Keywords

actomyosin ring; septum; yeast; membrane trafficking; constriction; cleavage furrow

Introduction

Cytokinesis, the final step in cell division, is essential for growth, development, and cell differentiation [1, 2]. After the nucleus divides by mitosis, the cell splits its cytoplasm into two via cytokinesis, giving rise to two daughter cells. Successful cytokinesis ensures that the two daughter cells maintain genomic integrity and chromosome number during division. In animal and fungal cells, cytokinesis occurs via the constriction of an actomyosin based contractile ring to form the cleavage furrow [3]. The fission yeast, *Schizosaccharomyces pombe,* is an excellent model system for understanding the molecular mechanism for actomyosin ring formation [4]. However, cytokinesis in fission yeast is more complex as it also requires the formation of a septum (cell wall) at the division site in addition to assembling a contractile actomyosin ring. Upon mitotic commitment, the cell prepares for cytokinesis through the recruitment of proteins necessary for ring formation to the precursor nodes [5, 6]. The Septation Initiation Network (SIN) pathway, activated by the GTPase Spg1, promotes proper positioning of the precursor nodes to the cell middle surrounding the nucleus to ensure that the actomyosin ring forms between the incipient daughter nuclei, thus preventing polyploidy and aneuploidy [4, 7] . This ensures that the cell divides along the middle, thereby generating two equal sized daughter cells.

The proteins that are recruited to the precursor nodes prior to initiation of cytokinesis, and their dynamics, have been assiduously described in several studies [5, 6, 8–11]. Once the precursor nodes recruit the formin Cdc12, actin nucleation and filament formation occur [5, 12, 13]. Different models have addressed how the actomyosin ring is assembled during cytokinesis; one model proposes that the type II myosin interacts with actin filaments using a search, capture, pull, and release mechanism to form a ring (Figure 1Ai) [8]. An alternate model, that is not mutually exclusive, proposes that the ring assembles from a single site, where two groups of parallel actin filaments of opposing direction rearrange to form a structure with mixed directionality (Figure 1Aii) [14]. A third model proposes that non-medial actin cables generated mainly by the formins Cdc12 and to an extent For3 are incorporated into the cells medial region to condense into a ring (Figure 1Aiii) [15, 16]. The SIN pathway works in parallel with the spindle assembly checkpoint to ensure that constriction initiates only after initiation of chromosome segregation [11, 17–21]. Unlike most mammalian cells where ring constriction initiates immediately upon ring assembly, in fission yeast the ring undergoes a maturation or dwell phase following its assembly in early anaphase A [22]. During anaphase B, the maturation phase ends, and ring constriction is initiated [11]. A recent report indicates that ring constriction occurs in two phases: a slow phase that initiates during anaphase B and a faster phase once anaphase completes [11, 23],.

The constriction of the ring is concurrent with septum deposition along the membrane adjacent to the ring [11, 24]. The septum, once built, physically separates the two daughter cells. It is comprised of a middle layer (primary septum) flanked by two secondary septa [25–27]. Several reviews extensively describe the molecular structure of the fission yeast septa [28, 29]. After completion of ring constriction, the septum matures, and the middle layer (primary septum) is degraded by digestive enzymes, marking the completion of cytokinesis [30, 31]. The secondary septum remains intact and serves as the new cell wall for the two daughter cells [27]. This process of septum synthesis during furrow formation is unclear and the molecular details of its spatiotemporal coordination with actomyosin ring constriction are poorly understood.

Studies on the regulation of cytokinesis in fission yeast have focused mostly on the structural aspects of the ring and how it is assembled [24, 32, 33]. Recently, super-resolution microscopy enabled the spatial mapping of proteins localized to the cytokinetic nodes and to the ring after assembly, showing their proximity to the ring and the membrane [34, 35]. However, once the ring assembles, how the organization of these proteins drive concurrent ring constriction and septum ingression remains unclear.

Septum driven cytokinesis in fission yeast.

In fission yeast, cytokinesis involves both ring constriction and simultaneous septum/cell wall ingression. The primary septum is mainly comprised of linear 1,3-β-glucan, polymerized by the β-glucan synthase Bgs1 (Table 1) [26, 36]. The secondary septum is structurally different from the primary septum and primarily consists of 1,6-β-branched 1,3-β-glucan, synthesized by Bgs4 (Table 1) [23, 27, 36, 37]. In addition, the primary and secondary septum also consists of α(1,3)-glucan synthesized by Ags1/Mok1 (Table 1) [38–40]. The glucan synthase homolog Bgs3 also localizes to the septum during cytokinesis and is speculated to contribute to the synthesis of 1,6-β-branched 1,3-β-glucan [41]. Bgs1–3 and Ags1 are integral membrane proteins and localize to the division site in a manner dependent on the actomyosin ring [23, 25, 27, 38, 39, 41–45]. Bgs1 has been shown to require the ring protein Cdc15 for its localization to the division site [43]. In *cdc15* mutants, where Bgs1 recruitment to the division site is impaired, the actomyosin ring is not properly anchored to the membrane and has been shown to slide [43, 46]. The integral membrane protein Sbg1 links the actomyosin ring with Bgs1 [47, 48]. It is unclear if Bgs1 enzyme activity is needed for anchoring the ring. While the authors report that addition of the drug Aculeacin A (blocks glucan synthesis) does not lead to ring sliding, Bgs1 is resistant to this drug and thus one cannot conclude if the Bgs1 enzymatic activity is necessary for anchoring the ring [49, 50]. Bgs1 has been reported to cooperate with Paxillin Pxl1 and Cdc15 to maintain ring integrity and to promote septum synthesis [51]. Similar to *cdc15* and *bgs1* mutants, *pxl1* mutants also show ring sliding defects [51]. Paxillin has been shown to recruit the phosphatase calcineurin Ppb1 to the division site, which in turn dephosphorylates Cdc15 and promotes Bgs1-dependent septum synthesis [52]. During anaphase the SIN pathway is activated and Bgs1 is recruited to the division site as the ring completes maturation and initiates constriction [11]. At this stage the rate of ring constriction and septum ingression is slow and only the primary septum with linear 1,3-β-glucan are synthesized [11]. Once anaphase completes, Bgs4 is also recruited to the division site and the rate of ring

constriction and septum ingression are accelerated [11]. It is possible that the acceleration in septum ingression is due to that fact that Bgs4 and Ags1 make 1,6-β-branched 1,3-β-glucan and α(1,3)-glucan respectively, that provide support and rigidity to the septum. Synthesis of a more rigid septum could lead to faster membrane invagination and ring constriction since the septum is coupled to the membrane barrier which in turn is associated with the actomyosin ring [27, 43].

Bgs1 enzyme activity promotes proper septum ingression and simultaneous ring constriction [26, 51, 53, 54]. Cells with hypomorphic *bgs1* or absence of *bgs1* assemble a normal ring but show delayed and slow furrow formation with severe defects in septum morphology [26, 51, 53–55]. It is unclear why septum deposition is required for timely initiation of ring constriction. One report suggests that cell-walled organisms such as fission yeast have high internal turgor pressure that antagonizes furrow formation [54]. This high internal turgor pressure is approximated to be ~0.95MPa, similar to the pressure in a racing bike tire [54]. Mathematical estimations suggest that actomyosin ring constriction does not generate sufficient force to overcome this turgor pressure [54]. Efforts to explain how the high internal turgor pressure is overcome during cytokinesis leave open questions. *In vitro* studies in yeast protoplasts indicate that constriction is dependent on type II myosin and ATP [56, 57]. However, *in vivo* experiments have shown that cytokinesis proceeds, even when the actomyosin ring is artificially removed using the actin depolymerizing drug Latrunculin- A [54]. In these Latrunculin-A treated cells, septum ingression must complete about 50% of the septum length, prior to removal of the contractile actomyosin ring for a complete septum to form. Interestingly, the rate of septum synthesis is slower in these cells, compared to untreated cells, indicating that the actomyosin ring is still necessary for efficient cytokinesis. Based on these observations the authors propose a model in which septum ingression provides the force required to overcome internal turgor pressure for membrane furrowing [54]. Actomyosin ring constriction defects reported in mutants with the thermo-sensitive *bgs1* allele *cps1–191* can also be explained by the fact that Bgs1 is required for anchoring the ring to the membrane and for actomyosin ring integrity [43, 51]. Anomalies in ring stability could also lead to constriction defects. Another consideration is that the actomyosin ring may fail to constrict when it is coupled to a rigid septum. Coupling of the septum to the membrane barrier could be detrimental to membrane ingression and ring constriction as the rigid septum cannot be pulled in during this process. In this scenario, the ring would constrict and form a membrane furrow only upon *de novo* septum deposition and ingression. Thus an alternate model suggests that under normal conditions, building the septum *de novo* towards the cell interior as the ring constricts, is necessary for septum ingression [58]. This is further supported by the observation that in *bgs4* mutants lacking secondary septum, the ring is no longer coupled to the primary septum and the rate of constriction is faster [27]. Moreover, contrary to the model proposed by Proctor *et al.*, [54] this report shows that the ring can constrict even in the absence of septum ingression suggesting that ring constriction *per se* does not need septum ingression. It is likely that septum ingression is only necessary when the membrane is coupled to the septum [27].

While septum ingression drives furrow formation, the actomyosin ring is required to spatially coordinate septum synthesis. By using microfluidic chambers to manipulate the shape of the actomyosin ring, it is possible to alter the rate of septum ingression [59]. In

regions of the ring with increased curvature, the rate of corresponding septum ingression is higher [59]. Moreover, in cells with misshapen actomyosin rings, curvature-dependent septum ingression over time corrects the shape of the cleavage furrow to a regular circular shape [59]. Thus, it is possible that septum ingression is activated by the actomyosin ring in a mechanosensitive manner. Evidence for mechanosensitive activation of septum synthesis has also been reported by another study [60]. A mathematical model was developed to explain how septum synthesis is spatially coordinated such that the shape of the furrow is fairly circular [60]. This model suggests that septum deposition responds to actomyosin ring tension, resulting in faster septum deposition in regions of the ring with high tension due to increased curvature [60]. Thus, throughout constriction, the septum synthesizing apparatus responds to the forces generated by the actomyosin ring and correspondingly synthesizes septum. The rate of furrow formation is set by the rate of septum synthesis, which is imposed by the force generated from the actomyosin ring [60]. This is also supported by the fact that type II myosin mutants, *myo2-E1* and *myp2Δ* show ring constriction defects and corresponding delays in furrow formation suggesting delays in septum ingression [61].

The contractile actomyosin ring also acts as a landmark for the septum synthesizing apparatus.

While the actomyosin ring is necessary to initiate septum synthesis, it is no longer necessary once septum synthesis initiates [62–65]. It has been reported that apart from providing a contractile structure, the ring has an additional and likely critical role in cytokinesis, where it acts as a landmark for the recruitment of the septum synthesizing apparatus (Figure 1B) [23, 25, 38, 39, 44]. During mitosis, the anillin-like protein Mid1 recruits proteins required for actomyosin ring formation to the cytokinetic nodes [6, 66–68]. Mid1 recruits the type II myosin Myo2, its regulatory light chain Rlc1, the myosin II light chain Cdc4 and the IQ-GAP Rng2 in a complex [6]. Mid1 also recruits the F-BAR protein Cdc15 to the nodes. Both these complexes then independently recruit the formin Cdc12 to the division site [6, 13]. Once the formin Cdc12 is recruited to the nodes it starts nucleation of actin filaments [22]. Assembly of the actomyosin ring is regulated at multiple levels. The Septation Initiation Network (SIN), analogous to the Hippo signaling pathway, couples ring constriction and septum formation with chromosome segregation [17, 69, 70]. The terminal SIN kinase, Sid2, phosphorylates Cdc12, thus promoting its F-actin bundling activity [18]. Thus timely recruitment of the proteins to the nodes and the activation of the SIN pathway ensure that the actomyosin ring starts assembly and is available for constriction once the cells start chromosome segregation [11]. The ring completes assembly during anaphase A but starts constricting only during Anaphase B (Figure 1B) [11, 22]. This delay in constriction could be due to the fact that after its assembly, the ring acts as a landmark to recruit proteins required for septum formation, and constriction only initiates once these proteins are available to start septum synthesis. Indeed, during anaphase B ring constriction is slow when Bgs1 is the only septum synthesizing enzyme recruited (Figure 1B) [11]. Upon telophase onset, Bgs4 is recruited and constriction occurs at a faster rate (Figure 1B) [11]. A key protein involved in recruiting or stabilizing proteins at the actomyosin ring is the F-BAR domain containing protein Cdc15 [42, 46]. The BAR domain of Cdc15 interacts with the membrane, while the SH3 (Src homology 3) domain binds proteins that associate with the

ring [42, 46, 71]. Thus Cdc15 is required for anchoring the actomyosin ring to the membrane [34, 43]. The SIN pathway kinase Sid2 also phosphorylates Cdc14, the Clp1 phosphatase which dephosphorylates Cdc15 [19–21]. Phospho-regulation of Cdc15 results in a conformational change that promotes its oligomerization and enables it to scaffold numerous proteins at the interface between the plasma membrane and the actomyosin ring [19]. While Cdc15 is required for cytokinesis, it is not essential for actomyosin ring formation in the presence of an active SIN pathway as observed in *cdc15Δ* and *cdc15–140* mutants [72, 73]. However, actomyosin rings are highly unstable and disintegrate in *cdc15–140* mutants suggesting that it is required for ring maintenance [38, 72, 73]. The actomyosin ring in cells lacking *cdc15* may not be competent to undergo constriction [43, 72]. This is due to two factors: (i) misregulation of Cdc12, discussed above, and (ii) failure to recruit the septum synthesizing apparatus [23, 25, 38, 39, 43, 44].

Once the ring assembles, Cdc15 levels at the division site continue to increase [74]. Cdc15 via its SH3 domain scaffolds numerous proteins to the division site [42]. The SH3 domain is also required for the localization of Bgs1 to the actomyosin ring [43, 51]. The catalytic activity of glucan synthases requires activation of the Rho GTPase, Rho1 [37, 75]. Cdc15 interacts with the Rho1 GEF (guanine nucleotide exchange factor) Rgf3 and helps recruit it to the division site after actomyosin ring assembly [42, 76–80]. In addition to Cdc15, Imp2, another F-BAR and SH3 domain containing protein, is involved in scaffolding proteins at the actomyosin ring [42, 46]. Cdc15 and Imp2 SH3 domains function in a redundant manner. The SH3 domains of these two proteins function cooperatively and mutants lacking both the SH3 domains are not viable [46]. In *cdc15ΔSH3 imp2ΔSH3* double mutants, analysis of the spores indicate that these mutants display fragmented actomyosin rings and fail cytokinesis [46]. Furthermore, tandem affinity purification of the SH3 domains and subsequent mass spectrometry identified multiple proteins that associate with either Cdc15-SH3 or Imp2-SH3 [42]. Thus, the SH3 domains structurally stabilize the actomyosin ring and function as a scaffold that recruits multiple proteins to the ring after assembly. Interestingly, while Cdc15 localizes to the division site prior to onset of actomyosin ring assembly, the primary septum synthesizing apparatus including Bgs1 and Rgf3 localize to the division site only after the ring is assembled [11, 45, 51, 76, 77]. This ensures that septum synthesis does not initiate before chromosomes start segregation. We have shown along with others that Cdc15 levels at the ring rapidly increase after ring assembly, and it is possible that the Cdc15 population recruited after ring assembly specifically promotes localization of Bgs1 and Rgf3 [42, 74, 81]. Bgs1 localization at the membrane also requires the small GTPase Cdc42 [45, 82]. It has been reported that Cdc42 is activated at the division site as the ring completes assembly [45, 83]. Cdc42 activation at the assembled ring depends on the GEF, Gef1, and mutants lacking *gef1* display a delay in Bgs1 localization to the ring [45]. It is possible that while Cdc15 is present at the ring from the onset of ring assembly, Bgs1 is recruited to the ring only upon Gef1-mediated Cdc42 activation in the assembled ring [45]. The septum ingresses centripetally indicating that deposition occurs uniformly along the membrane behind the actomyosin ring and along the ingressing membrane. This indicates that Cdc15 and thus the septum building apparatus is uniformly localized along the actomyosin ring leading to centripetal septum deposition [23, 25, 27, 38, 41, 44, 81].

Another F-BAR domain containing protein, Rga7 has been shown to be required for maintaining actomyosin ring integrity, and for proper septum synthesis in cooperation with Cdc15 and Imp2 [84]. Rga7 is a Rho2 GTPase activating protein (GAP), but the catalytic activity is not required for its role in cytokinesis [84, 85]. Rga7 interacts with Rng10, a coiled-coil protein that enables it to interact with the membrane at the division site and promote ring stability [86]. Rga7 has been shown to specifically promote Bgs4 recruitment to the division site [87]. The integral membrane protein Sbg1 has also been shown to promote Bgs1 localization at the septum [47, 48]. Bgs1 and Sbg1 localization are dependent on each other and Sbg1 associates with both Bgs1 as well as the actomyosin ring components thus linking the two [48].

Membrane trafficking events during cytokinesis

Recent reports indicate that Membrane trafficking events at the division site are essential for cytokinesis [45, 88]. Blocking endocytosis using the Arp2/3 inhibitor CK-666 prevents initiation of septum ingression and cell separation during cytokinesis [81]. Interestingly, CK-666 treatment does not inhibit actomyosin ring formation [81]. This suggests that membrane trafficking events are involved in distinct cytokinetic events. The trafficking events participating in the different cytokinetic stages are discussed below.

Actomyosin ring maturation.

After the ring assembles, the Bgs1 is delivered to the membrane adjacent to the ring via actin-mediated delivery (Figure 2A) [11, 45]. The type V myosins have been shown to be required for this process [89]. Actin-mediated delivery depends on the formin For3 that nucleates actin to form cables [90]. It is not clear if For3 is also required for the delivery of glucan synthases. Mutants of *for3* only display minor cytokinetic defects suggesting that redundant delivery mechanisms could function here [16]. However, *for3* mutants combined with an activated allele of the formin *cdc12* is lethal, but in this case the cells fail to assemble an actomyosin ring [16]. Bgs1 localization is also dependent on the F-BAR Cdc15 and the timely activation of the GTPase Cdc42 [45, 91]. Upon blocking endocytosis by CK-666 treatment, the cells form a ring but fail to recruit Bgs1. In addition, the levels of Cdc15 which typically increases at this stage, remains the same [81]. Similar observations were made with the endocytosis defective *arp3-c1* cold sensitive mutant [81]. At the maturing ring, Gef1-mediated Cdc42 activation facilitates recruitment of Bgs1 to the ring [45]. On the other hand, Bgs1 delivery to the ingressing membrane depends on Scd1-mediated Cdc42 activation [45]. While the mechanism by which Cdc42 promotes Bgs1 delivery is not clear, *gef1* mutants which do not activate Cdc42 during ring maturation show defects in type V myosin, Myo52 localization to the division site and fewer non-medial actin cables [45]. Therefore, it is possible that Cdc42 regulates actin-mediated delivery of Bgs1.

It has been shown that Gef1 mediated Cdc42 activation promotes endocytosis [81]. In *gef1* mutants, Cdc15 levels at the endocytic patches are higher and the patch itself displays longer lifetimes [81]. In a *gef1Δ* mutant combined with an activated allele of *cdc12*, *cdc12Δ503*, the cells show defects in septum ingression and do not constrict centripetally [81]. In these *gef1Δcdc12Δ503* mutants, the cells display irregular distribution of Cdc15 along the ring

such that regions of the ring with increased Cdc15 levels constrict at a faster rate. Since Cdc15 recruitment to the assembled ring requires endocytosis [81] and Cdc15 is involved in endocytosis [92], it is possible that after ring assembly, the cell recruits Cdc15 to the ring mainly from the endocytic patch. In agreement, disruption of endocytosis leads to defects in Cdc15 recruitment resulting in its irregular distribution at the ring [81]. These findings indicate that membrane trafficking events such as endocytosis are required to uniformly organize Cdc15 along the ring thereby leading to centripetal septum ingression and constriction.

Actomyosin Ring Constriction and Septum Ingression.

After ring constriction initiates, glucan synthases such as Bgs4 and Ags1 are recruited to the membrane adjacent to the ring and to the ingressing membrane and rate of ring constriction accelerates (Figure 2B) [11, 23, 38, 39]. While Ags1 is recruited during late Anaphase B, Bgs4 is recruited during telophase [11]. Surprisingly, while Cdc42 is required for Bgs1 recruitment, it does not promote recruitment of Bgs4 [45, 91]. It is unknown if Cdc42 is also required for the delivery of Bgs3 and Ags1. Thus, Cdc42 appears to promote delivery of specific cargoes and may not simply regulate actin cable formation during cytokinesis.

Another component of the membrane trafficking machinery is the TRAPP-II delivery complex which has been shown to participate in cytokinesis [88]. This complex not only delivers glucan synthases to the division site, but during ring constriction, it also provides additional membrane via the delivery of vesicles and tubulovesicular structures [88]. The TRAPP-II complex localizes all along the ingressing furrow and is suggested to be primarily required for delivery of membrane necessary for membrane expansion during this process (Figure 2B) [88].

The organization of the trafficking machinery is spatially regulated during cytokinesis. While the TRAPP-II complex promotes tethering and fusion of vesicles along the entire ingressing membrane, the exocyst contributes to delivery only at the rim of the cleavage furrow [88]. It is possible that this spatial segregation of trafficking apparatus facilitates delivery of specific cargoes to different regions of the cleavage furrow. It has also been reported that endocytosis is mainly confined to the rim of the cleavage furrow [88]. It is not clear whether endocytosis is limited to the rim to ensure unhindered membrane expansion at the leading edge of the cleavage furrow, or whether it is required for the redistribution and/or recycling of proteins as cytokinesis progresses.

Septum digestion and cell separation.

After septum formation, septum digesting glucanases are delivered between the outer edge of the primary septum and the lateral cell wall to properly digest the primary septum and allow cell separation (Figure 2C, Table 1) [30, 31, 93–98]. The delivery of the glucanases are dependent on the exocyst complex [93, 94, 99, 100]. The exocyst complex is essential for viability, however temperature sensitive *sec8–1* exocyst mutants and germinated spores of deletion mutants show polarized cell shape, indicating that it is not vital to cell polarity [93, 101]. Similarly, *sec8–1* mutants under restrictive conditions display a septum, indicating that the exocyst is dispensable for septum formation [93]. However, these mutants fail to

localize the glucanases such as Eng1 and Agn1 to the division site, and as a result do not undergo cell separation [94]. Thus, the primary role of the exocyst complex during cytokinesis is the delivery of glucanases necessary for cell separation.

In addition to the exocyst complex, delivery of the hydrolytic enzymes required for septum digestion also depends on the Rho3/4 GTPases [93, 99, 100, 102]. Rho3 promotes the delivery of secretory vesicles to the delivery site, likely through interaction with the exocyst complex [99]. Interestingly, overexpression of *rho3*$^{+}$ but not *rho4*$^{+}$ can suppress the separation defects of exocyst mutants [99, 100]. However, overexpression of *eng1*$^{+}$ or *agn1*$^{+}$ suppresses the *rho4Δ* multi-septation phenotype [103]. *rho4Δ* cells display secretory defects as they exhibit reduced levels of Eng1 and Agn1 in cell culture, and fail to localize these enzymes to the septum at elevated temperatures [103]. When exocyst functionality is compromised, Rho3 localization to the division site is impaired [99]. In contrast, Rho4 localization is independent of the exocyst complex but the exocyst subunit Sec8 fails to localize to the division site in *rho4* mutants [100]. The Rho GEF, Gef3 also contributes to cell separation, likely via Rho4 activation [102]. Interestingly, cell separation defect is elevated in *gef3Δgef1Δ* double mutants [102]. It is not clear if Gef1-mediated Cdc42 activity is also required for cell separation [102].

Septin protein complexes are also required for the delivery of glucanases to the division site. This includes the anillin homolog Mid2 and the septin proteins Spn1–4 [94, 98, 100, 102, 104–109]. Mid2 organizes the septin ring that forms on either side of the septum barrier [106]. In the absence of the septin ring or *mid2*, the septum digesting glucanases are not localized properly to the base of the primary septum [94]. The localization of the septin ring and the exocyst complex at the division site requires Rho4 GTPase [100]. The septin and exocyst localizes the GEF Gef3 which in turn activates Rho4 [102, 105]. It is tempting to speculate that the septins may act as a landmark to guide Rho4 activation, to ensure that the fusion of vesicles containing the glucanases Agn1 and Eng1 occurs only at the base of the primary septum to promote cell separation and prevent cell lysis. Expression of Mid2 as well as the glucanases is regulated by the transcription factor Ace2 in a cell cycle dependent manner thus ensuring that cell separation occurs after septation [110, 111].

These different trafficking events occur in a temporally sequential manner at distinct regions of the division site. Membrane trafficking events during cytokinesis are thus tightly regulated spatiotemporally to coordinate different cytokinetic events.

Future Avenues

In fission yeast, the septum plays an important role in cytokinesis and proper septum formation and cell separation are necessary for cell integrity and viability. By acting as a landmark for septum synthesis, the ring ensures that the septum is always built adjacent to the actomyosin ring, thus maintaining the fidelity of the division site positioning. As described above, membrane trafficking events are critical for cytokinesis. Moreover, proper cytokinesis requires spatiotemporal regulation of different membrane trafficking events. Recent advances raise several intriguing questions in the field. Failure in any one of the cytokinetic steps can lead to cell death. Furthermore, cytokinetic events need to occur in the

proper and sequential manner in order to maintain cell integrity and successful cell separation. How does the cell precisely organize the different events during cytokinesis? Evidence indicate that signaling pathways including the SIN pathway and the Cdc42/Rho GTPases regulate the different steps in cytokinesis [17, 45, 69]. How are these different signaling pathways regulated to ensure that each step during cytokinesis occurs in the correct order and in coordination with nuclear division? How are Cdc42 and Rho GTPases regulated to organize cytokinesis events? During cytokinesis, the septum building apparatus is deposited at the ring membrane interface and also to the ingressing membrane. Membrane deposition occurs at the ingressing membrane barrier and digestive enzymes are delivered to the rim of the membrane barrier. How do signaling pathways spatially restrict these processes to distinct sites of action to prevent ectopic cell wall deposition and cell lysis? Addressing these questions will provide a better understanding of the mechanistic details of cytokinesis and provide insights into how the cell regulates multiple membrane trafficking-mediated polarization events. Thus, knowledge gathered on the mechanistic details of membrane trafficking and polarization during cytokinesis will provide a paradigm for understanding fundamental principles of cell polarity in general.

The cell wall is analogous to extracellular matrix in higher eukaryotes. Interestingly, extracellular matrix remodeling has been shown to be required for cytokinesis in other systems. In *C. elegans* embryos, germ cells, and in pre-implantation mouse embryos, cytokinesis requires extracellular matrix remodeling [112–115]. In *C. elegans*, a defect in chondroitin biosynthesis results in the failure to initiate cytokinesis during early embryogenesis [114]. In another study it was shown that extracellular hemicentins localize to the cleavage furrow in the germline of *C. elegans* and also in mouse embryos [112]. In mutants lacking hemicentin, the cleavage furrow initiates but later regresses, leading to cytokinetic failure [112]. It is unclear how these extracellular matrix components contribute to cytokinesis. A more recent study has also shown that optogenetically activated RhoA at the medial region of *C. elegans* embryo initiates actomyosin ring constriction but fails to complete this process [116]. It is unclear why the cleavage furrow in these cells fail cytokinesis. One explanation could be that premature initiation of the cleavage furrow by RhoA activation fails because the extracellular matrix is not yet properly remodeled to facilitate cytokinesis. Thus, coupling of extracellular matrix remodeling and actomyosin ring mediated furrow formation may be a prevalent feature of cytokinesis in eukaryotes. Further investigations will reveal why and how extracellular matrix promotes cytokinesis in other organisms.

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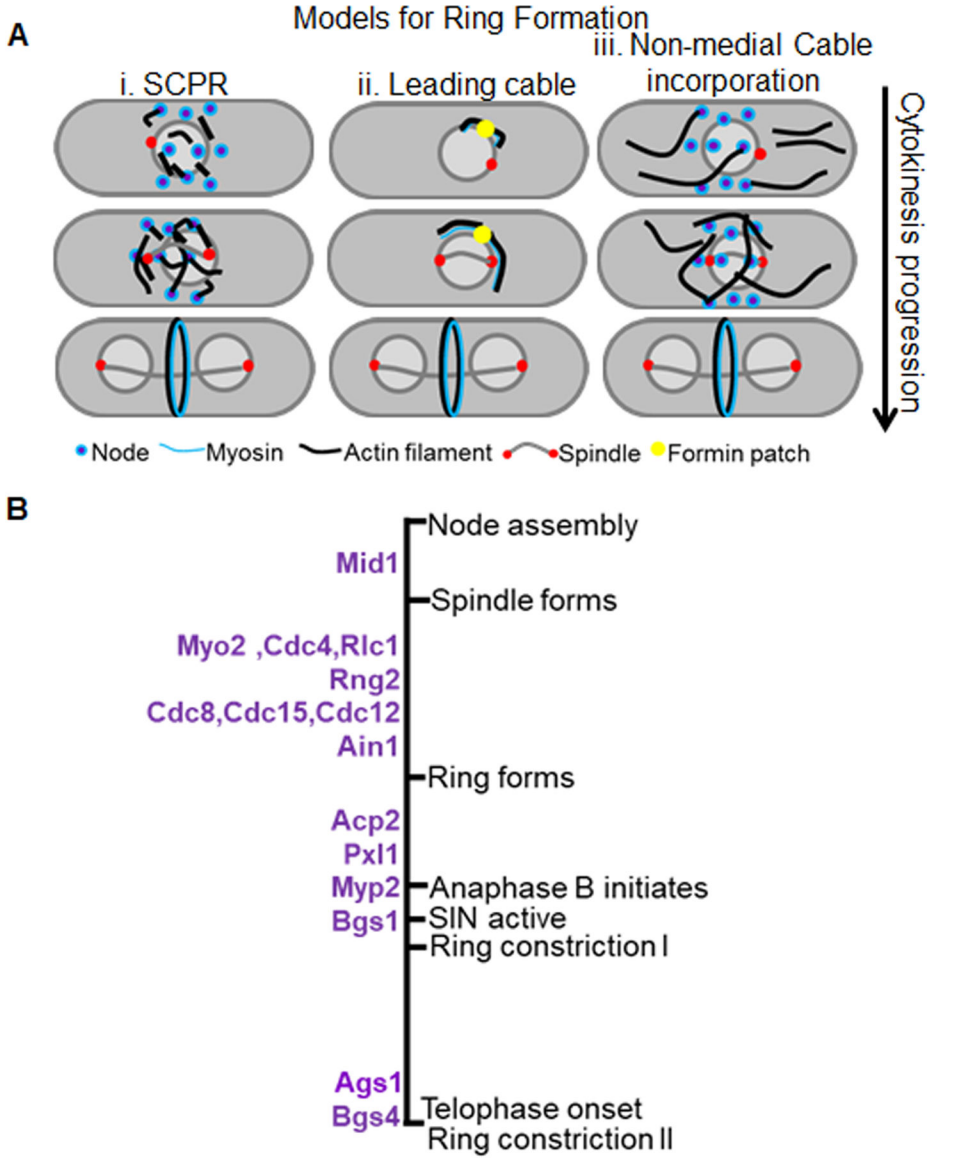

Figure 1. Early cytokinetic events.

A. Models for actomyosin ring assembly. i. The search-capture-pull-release model- the formin Cdc12 when recruited to the nodes nucleates actin while the type 2 myosin binds actin and pulls on the filaments to coalesce into a ring. ii. The Leading cable model- A patch of formin, Cdc12, nucleates actin cables that extends along the periphery of the division site to eventually form a ring. iii. Non-medial cable incorporation model. The formins Cdc12 and For3 nucleate actin to form non-medial cables that are then incorporated into the cells medial region to for a ring. **B.** Timeline of key proteins recruited to the division site leading to ring constriction with reference to different cytokinetic steps and mitotic progression. Biphasic ring constriction is depicted as Ring constriction I that occurs during anaphase B after Bgs1 recruitment and is slow and as Ring constriction II that occurs during telophase after recruitment of Ags1 and Bgs4 and is accelerated.

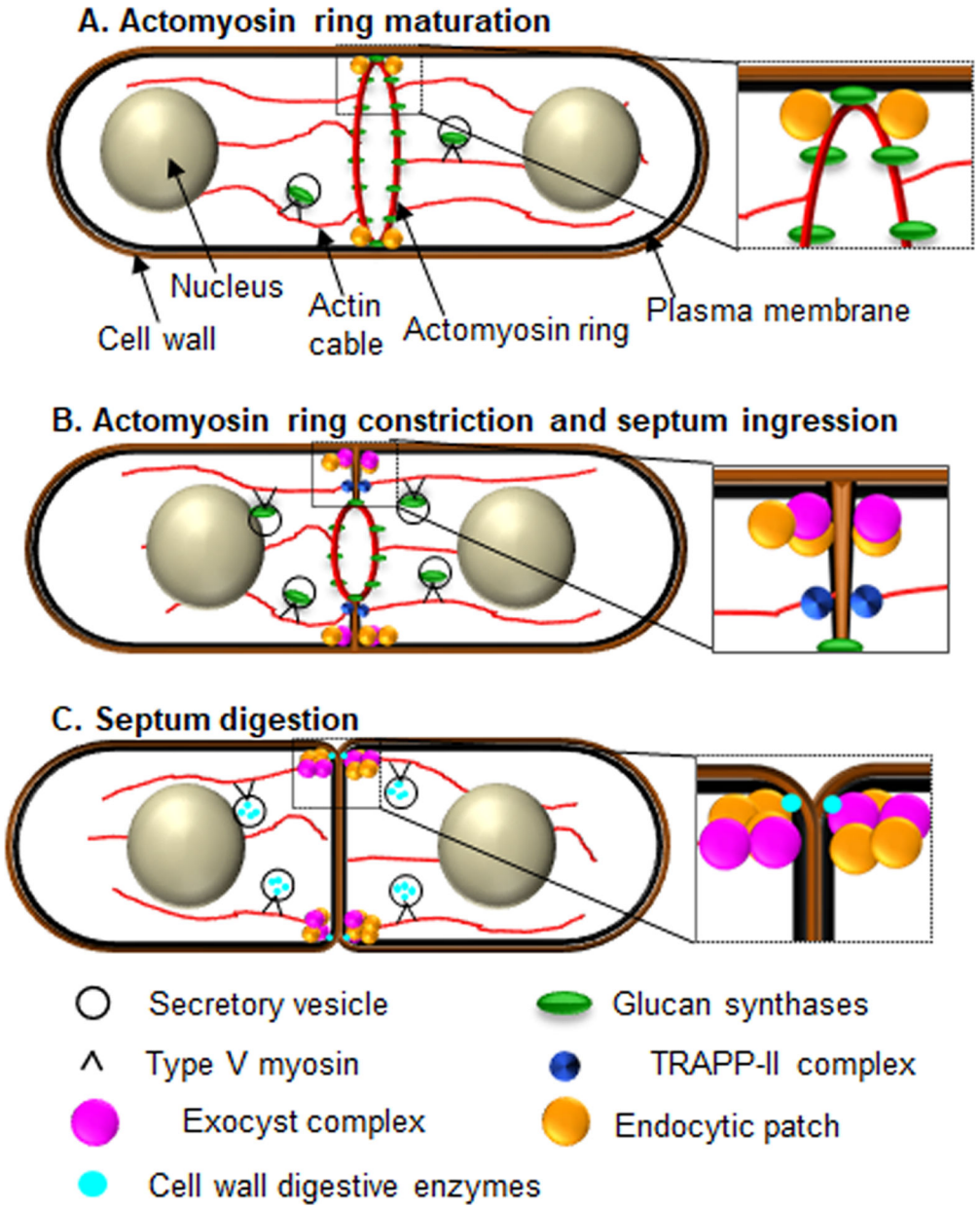

Figure 2: During cytokinesis membrane trafficking events promote septum formation, membrane ingression and septum digestion in fission yeast.

A. Actomyosin ring Maturation: During actomyosin ring maturation the septum synthesizing glucan synthase Bgs1 is delivered to the membrane adjacent to the ring through secretory vesicles and type V myosin, and via the TRAPP-II complex. **B.** Actomyosin ring constriction and septum ingression: Ring constriction occurs in a biphasic manner. In Anaphase B the actomyosin ring constriction initiates at a slow rate and this is concurrent with septum ingression. In late anaphase B Ags1 is delivered while Bgs4 is delivered after the completion of Anaphase B. At this stage the rate of constriction and septum ingression accelerates. Glucan synthases delivery and membrane deposition continues during ring constriction and septum ingression. Endocytosis and the exocyst complex is mainly restricted to the rim of the ingressing membrane. **C.** Septum digestion: Cell wall digestive

glucanases are delivered to the base of the primary septum via exocyst-mediated delivery leading to cell separation.

Table 1:

Enzymes delivered to the division site during cytokinesis.

Enzyme	Polysaccharide	Septum structure	Localization depends on	Reference
Septum synthesis				
Ags1/Mok1	α(1,3)-D-Glucan	Primary septum and secondary septum	Exocyst complex, Pck2	[38, 39]
Bgs1	Linear β(1,3)-D-Glucan	Primary septum	Sbg1, SIN pathway, Cdc15, Cdc42, Myo52, TRAPP II complex	[25, 26, 43, 45, 47, 51, 88, 89]
Bgs4	Branched β(1,3)-D-Glucan	Primary and secondary septum	Rga7, TRAPP II complex	[23, 87]
Septum digesting				
Agn1, Eng1	endo-1,3-alpha-glucosidase	Between the base of the primary septum and the outer cell wall	Rho3, Rho4, Exocyst complex, Septin ring	[30, 31, 93, 95–97, 99, 100, 102, 103, 105, 117, 118]