Review

Processes Controlling the Actin-Myosin Contractile Ring During Cytokinesis in Fission Yeast

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Processes Controlling the Actin-Myosin Contractile Ring during Cytokinesis in Fission Yeast

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Abstract: Cytokinesis, as the last stage of the cell division cycle, is a tightly controlled process amongst all eukaryotes, and defective division can lead to severe cellular consequences, and is implicated in serious human diseases and conditions such as cancer. Both mammalian cells and the fission yeast *Schizosaccharomyces pombe* use binary fission to divide into two equal sized daughter cells. In *S. pombe* such cytokinetic division is driven by the assembly of an actin-myosin contractile ring (ACR) at the cell equator between the two cell tips. The ACR is composed of a complex network of membrane scaffold proteins, actin filaments, myosin motors, and other cytokinesis regulators. Contraction of the ACR leads to the formation of a cleavage furrow which is severed leading to the final cell separation during the last stage of cytokinesis, abscission. This review describes recent findings defining the three phases to establish cytokinesis in *S. pombe*: ACR assembly, ACR constriction, and its coordination with septation. Collectively, we provide an overview of current understanding of the mechanisms regulating the ACR-mediated cytokinesis in *S. pombe*.

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1. Introduction

1.1. The Use of Fission Yeast to Study Eukaryotic Cytokinesis

Both mammalian cells and the fission yeast *Schizosaccharomyces pombe* use binary fission to divide medially. Fission yeast cells are encased in a cell-wall structure giving them their rod shape following growth by tip extension and divide equatorially. Therefore, the species is considered an excellent model organism for studying eukaryotic cytokinesis, where similar cellular processes occur.

Research using *S. pombe* has allowed the identification of many important conserved cell cycle regulators. As the mechanisms of assembly and constriction of the actin-myosin contractile ring (ACR) in *S. pombe* are very similar to those seen in mammalian cells, our current understanding of eukaryotic cytokinesis stems extensively from studies in fission yeast (Pollard and Wu, 2010).

During the 1970s, the work of Leland Hartwell and colleagues with the budding yeast *Saccharomyces cerevisiae* led to the discovery of a large number cell division cycle (*CDC*) mutants, and for the first-time eukaryotic genes required for cell division were characterized (Hartwell *et al.*, 1970, Hartwell *et al*., 1973). Study of the cell division cycle continued in the distally related fission yeast *S. pombe* by Paul Nurse and colleagues, with the discovery of equivalent *cdc* gene mutants (Nurse, 1975, Nurse *et al.*, 1976).

Later in the 1990s, two landmark reviews discussed aspects of the *S. pombe* cell cycle including the timing of events leading to cytokinesis, cell division, and mechanisms for determining the medial or equatorial division plane. At that time the *cdc16* and *cdc2* genes were thought to act as a molecular switch regulating *S. pombe* mitosis and cytokinesis (Chang and Nurse, 1993), and it was proposed that the division plane was determined by the position of the nucleus (Chang and Nurse, 1996). Subsequent research offered a deeper understanding of the *S. pombe* cell cycle regulation, including aspects of cytokinesis (reviewed in Nurse, 2020), and cell polarity (reviewed in Chang *et al*., 2019).

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Establishment of cytokinesis is mediated by a cytokinetic ACR that leads to the final separation of two daughter cells. In this short review, we describe *S. pombe* cytokinesis starting from the medial positioning of the division plane and the assembly of the cytokinetic ACR (Section 2), to the forces that generate tension and lead to constriction of the ACR, and the final separation of the two daughter cells (Section 3).

2. Actin-Myosin Contractile Ring (ACR) Assembly in Fission Yeast

2.1. Positioning of the Cell Division Plane

In *S. pombe* cellular growth occurs throughout a longer interphase period with this ceasing during the shorter mitosis and cytokinesis periods, after a certain cell length is achieved. During the cell cycle, "middle" and "end" or "tip" locations are specified by two spatial axes. The "middle" location is defined by the nucleus which is positioned at the cell centre by a microtubule-pushing mechanism where a force produced by the cytoplasmic microtubule bundles and is applied to the nucleus (Tran *et al*., 2001, Piel and Tran, 2009). Furthermore, this force is able to efficiently re-centre nuclei of cells exposed to nuclear displacement (Daga *et al*., 2005, Daga *et al*., 2006). The dynamic interplay between the nucleus and the microtubule cytoskeleton is illustrated and reviewed by Gallardo *et al*. (2019). Alternatively, the "end" or "tip" location is defined by formin-mediated actin assembly mechanism at cell tips (Martin *et al*., 2005), and polarity factors including the DYRK kinase Pom1p secreted in a gradient manner at cell poles. Additionally, Pom1p controls mitotic entry by phosphorylating the ACR scaffold protein Cdr2p at the cell "middle" through phosphorylating its membrane-binding C-terminal region (Gerganova *et al*., 2019).

Much evidence shows that the Anillin-like protein Mid1p localizes to the "middle" location and initiates ACR assembly (Paoletti and Chang, 2000, Almonacid *et al*., 2011, Saha and Pollard, 2012, Rezig *et al*., 2021). Mid1p has two membrane binding domains, the pleckstrin homology domain (PH) and the cryptic domain (C2) (Chatterjee *et al*., 2019). However, it only binds the plasma membrane after it is activated and resealed from the nucleus (Bähler *et al*., 1998, Almonacid *et al*., 2011). The roles of Mid1p in positioning the ACR are now well understood in *S. pombe*, and are reviewed in Rezig *et al*. (2022), with medial positioning of the ACR described in Figure 1.

In contrast, comprehension of the organization of proteins that assemble the complex cytokinetic machinery during cytokinesis is still relatively rudimentary. In *S. pombe*, cytokinesis proteins are recruited to the cell centre pre-determining the future division plane, these organized as cortical spots, called "nodes" (Akamatsu *et al*., 2014, Laplante *et al*., 2016). The next Section will discuss the nature of these nodes including their constituent proteins and spatiotemporal organization.

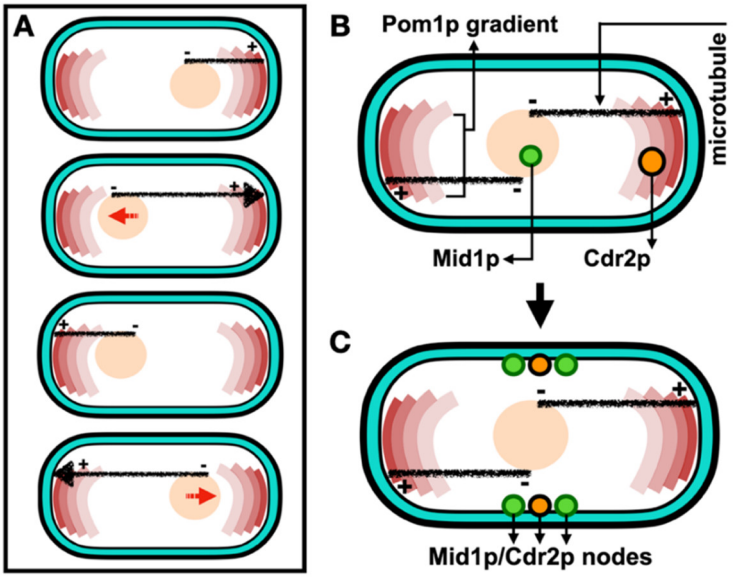

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Figure 1. Medial Positioning of the ACR in Fission Yeast. (**A**) Upon microtubule–cortex contact, polymerization at the microtubule plus end generates a pushing force (large arrowhead) that displaces the nucleus in the opposite direction (nuclear movement demonstrated by red arrows). The antiparallel direction of the microtubule bundle ensures that, over time, the nucleus oscillates back and forth toward the centre. (**B**) Mitotic entry is controlled by Pom1p phosphorylation of Cdr2p (this prevents Cdr2p from binding the plasma membrane and formation of cortical nodes) and Mid1p nuclear localization; in both cases the two scaffolding proteins are restricted from the cell equator. (**C**) Upon mitotic entry, both active Mid1p and Cdr2p scaffold the formation of cortical nodes. References within the text.

2.2. Molecular Organization of Nodes within the ACR

The current model for the ACR assembly includes two types of interphase nodes: type-1 "stationary" nodes containing Cdr1p, Cdr2p, Wee1p, Mid1p; and type-2 "anchoring" nodes containing Blt1p, Gef2p, Cdc15p, Rng2p, and Cdc12p (Zhu *et al*., 2013, Akamatsu *et al*., 2014). The "anchoring" nodes form the units that anchor the ends of myosin-II tails to the plasma membrane, with myosin-II heads extending into the cytoplasm. These nodes then merge into a ring-like structure, called the actin-myosin contractile ring (ACR) and, as its name implies, it is composed of actin filaments and myosin-II motors in addition to various classes of cytokinesis proteins (Malla *et al*., 2021).

Live cell imaging, high-speed fluorescence photo-activation localization microscopy (FPALM), and fluorescence resonance energy transfer (FRET) have shown to be excellent methods to dissect ACR nodes. They have revealed that nodes are discrete units with stoichiometric ratios and specific distribution of constituent proteins (Laplante *et al*., 2016, Akamatsu *et al*., 2017, McDonald *et al*., 2017, Malla *et al*., 2021). Furthermore, the localization of the ACR constituents is thought to be arranged in several layers relative to the plasma membrane, starting with plasma membrane-binding proteins and the tail of myosin-II, to intermediate cytokinesis proteins, and farthest from the plasma membrane lays myosin motor domains, F-actin and its cross-linkers (McDonald *et al*., 2017). Advances in laser scanning microscopy, such as Airy-scanning using very low laser power to acquire high-quality images, increased the resolution and signal-to-noise ratio and enabled the detection and measurement of even feint individual cytokinesis nodes (Sayyad and Pollard, 2022). Coalescence of nodes leads to the ACR assembly through the search capture pull and release (SCPR) mechanism, whereby Cdc12p nucleates actin filaments as Myo2p pulls actin filaments, thus producing the force required to pull the individual nodes into the ACR (Vavylonis *et al*., 2008, Laplante *et al*., 2016, Zimmermann *et al*., 2017). Such assembly of the ACR from node precursors is described in Figure 2.

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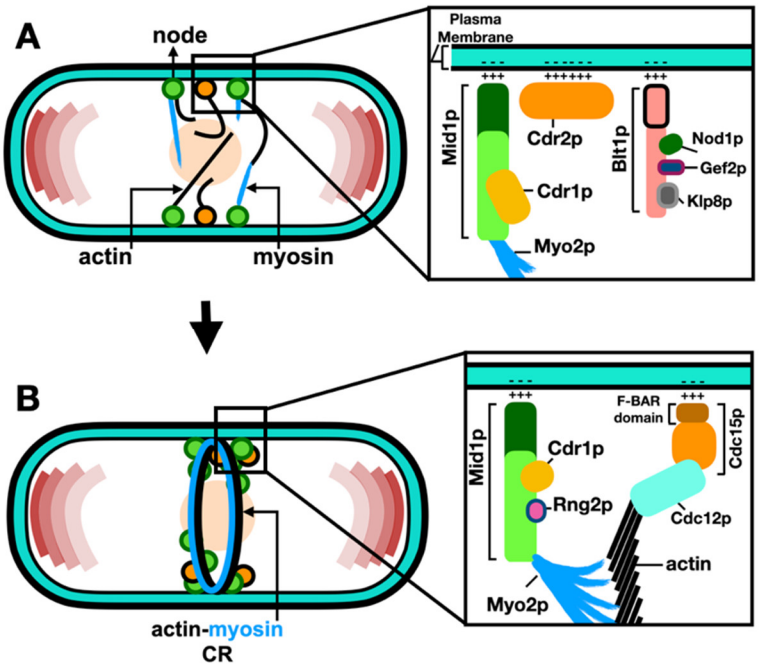

Figure 2. Assembly of the ACR from Node Precursors. (**A**) During interphase, type-1 and type-2 nodes orchestrated by Mid1p, Cdr2p, and Blt1p bind the plasma membrane and scaffold other proteins. (**B**) Coalescence of interphase nodes results in cytokinesis nodes. These mature nodes in turn recruit Cdc12p which nucleates actin filaments and interaction between Myo2p, with actin leading to the formation of the ACR. References within the text.

2.3. Anchorage of the ACR to the Plasma Membrane

After ACR assembly, Myo2p tails and Cdc15p localize to the plasma membrane, with the Myo2p heads, Myp2p and the bundle of actin filaments localizing 60 nm away from the plasma membrane (Swulius *et al*., 2018). It is suggested that this organization connects the bundle of actin filaments to the plasma membrane (Bellingham-Johnstun *et al*., 2021). Cdc15p next recruits Cdc12p to the ACR, and this interaction is thought to be essential for the ACR organization and stability (Snider *et al*., 2020).

The phospho-status of Cdc15p influences its ability to bind the plasma membrane, with phosphorylation of Cdc15p by Pom1p inhibiting its binding to the plasma membrane at cell tips (Bhattacharjee *et al*., 2020). Additionally, the p21-activated protein kinase (Pak1p), another polarity kinase, was found to regulate the function of Mid1p and Cdc15p (Magliozzi *et al*., 2020). Cdc15p has three regulatory components: an N-terminal Fre/Cip4 homology Bin/Maphiphysin/Rvs domain (F-BAR), a medial intrinsically disordered region (IDR), and a C-terminal Src homology 3 domain (SH3). While the F-BAR domain enables protein oligomerization and concentration on the plasma membrane to scaffold protein assemblies resulting in membrane deformation (Snider *et al*., 2021), it was recently found that phosphorylation of Cdc15p induces the separation of the Cdc15p IDR region resulting in an inhibition of Cdc15p phase separation, and the formation of condensate on the plasma membrane (Bhattacharjee *et al*., 2023).

The role of Cdc15p during *S. pombe* cytokinesis was examined using laser ablation. The ACR recoils after being severed, however, this recoil profile was greater and slower in the ablated ACR of Cdc15-depleted cells, suggesting that loss of Cdc15p decreases stiffness of the ACR material (Moshtohry *et al*., 2022). Furthermore, another F-BAR protein, Imp2p, was found to contribute to the stiffness of the ACR (Bellingham-Johnstun *et al*., 2021).

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3. ACR Constriction and Septation in Fission Yeast

3.1. Constriction of the ACR

The endosomal sorting complex required for transport (ESCRT) machinery has a well characterized role in mammalian cytokinesis, where it induces membrane remodeling events to disassemble the constricted ACR (reviewed in Bhutta *et al*., 2014a). ESCRTs were initially characterized in *S. pombe* in the early 1990s for regulating vesicle-mediated protein sorting (Takegawa *et al*., 1995, 2003, Iwaki *et al*., 2007). ESCRTs were then found to be required for cytokinesis in *S. pombe* (Bhutta *et al*., 2014b); however, the precise role(s) of ESCRTs during cytokinesis in fission yeast remains unclear.

The ATPase ESCRT associated protein Vps4p plays a critical role in recycling ESCRT complexes: such an interaction is catalyzed during abscission and leads to the final "cutting" of the thin intracellular bridge connecting the two daughter cells. Vps4p has been suggested to interact directly or indirectly with the C-terminal domain of Mid1p, probably through the lipid-binding PH domain, and Mid1p localization to the nodes is disrupted in *vps4*Δ cells (Rezig *et al*., 2021). Interestingly, Mid1p leaves the ACR upon constriction through phosphorylation by Sid2p (Willet *et al*., 2019), and coordination between Vps4p and Sid2p might regulate Mid1p disassociation from the cell cortex; however, this hypothesis requires further investigation as Mid1p leaves the ACR before the onset of abscission, when Vps4p is activated (Rezig *et al*., 2021).

3.2. Septation Coordinates with ACR Constriction

Unlike in mammalian cells where cytokinesis only implies the formation of a medial ACR, cytokinesis in *S. pombe* also requires the formation of a cell wall-like structure called the septum, physically separating the two daughter cells, at the division site. After ACR assembly and constriction, the septation initiation network (SIN) mediates the synapses of the amphid defective (SAD) kinase, Cdr2p, dispersal from the cell cortex into the cytoplasm, and septation is coordinated with ACR constriction (Rincon *et al*., 2017). The septum is composed of three layers: a middle primary septum layer that is later digested at the end of cytokinesis, and two flanking secondary septa which remain intact to form the new cell walls of separated daughter cells (reviewed by Pérez *et al*., 2018 and Hercyk *et al*., 2019).

S. pombe septation occurs in three stages. First, deposition of the septum cell-wall structure material is carried out through membrane trafficking events where secretory vesicles deliver the septum beta-glucan synthase 1, Bgs1p (Onwubiko *et al*., 2021). Localization of cell-wall building enzymes including Bgs1p depends on both Cdc42p and Cdc15p (Wei *et al*., 2016, Campbell *et al*., 2022). Second, during anaphase B the ACR constricts at a slow rate as the septum ingression is initiated; however, after the delivery of the alpha-glucan synthase 1, Ags1p, and Bgs4p, the rate of constriction and septum ingression is increased (Cortés *et al*., 2018). Third, after the ACR constricts, exocytosis leads to the delivery of glucanases and digestion of the primary septum leaving the two daughter cells each with a new cell wall (secondary septa) (Pérez *et al*., 2015).

Septation is coupled to ACR constriction, and the interaction between Bgs1p and the ACR is mediated by the paxillin Pxl1p (Cortés *et al*., 2015). Furthermore, Pxl1p accumulation during septum formation is thought to be mediated by an interaction with both the N-terminal F-BAR (Snider *et al*., 2020) and C-terminal SH3 domains of Cdc15p (Bhattacharjee *et al*., 2020). The mechanism of engagement of Pxl1p to both distal domains of Cdc15p is not well understood. However, a recent study demonstrated this interaction and found that Pxl1p binds to the Cdc15p F-BAR cytosolic domain (Snider *et al*., 2022).

Proctor and colleagues (2012) found that beta-glucan synthesis in important for the ACR to overcome the high turgor pressure during cytokinesis, suggesting that other pathways might coordinate with ACR constriction. Recent research used a fission yeast mutant *cps1-191*, defective in 1,3-beta-glucan-synthase septum synthesis, that arrests with a non-constricting ACR, to test if high turgor pressure restricted ACR constriction in such mutants (Chew *et al*., 2020). This showed that a decreased turgor pressure inhibits the ACR constriction in *cps1-191* mutants, suggesting that the

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extracellular glycan network regulates the ACR constriction and septum ingression, and that subsequent remodeling of the extracellular components relieves this restriction and facilitates the ACR constriction. Septum synthesis and coordination with ACR constriction are described in Figure 3.

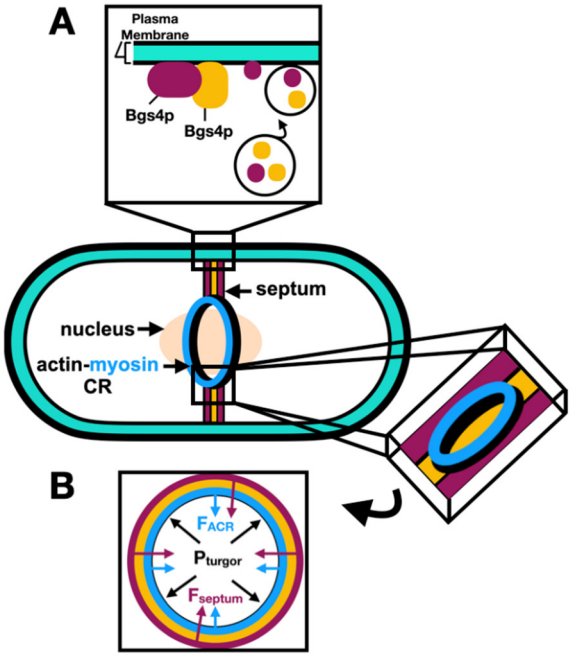

Figure 3. Septation is Coordinated with ACR Constriction. (**A**) Deposition of the septum material by membrane trafficking through secretory vesicles carrying the septum glucan synthases (Onwubiko et al., 2021). (**B**) Cross section of the deposited septum and constricting ring with internal turgor pressure (black), opposed by the inward forces of the ACR constriction (blue) and septation (purple) (Derived from Proctor et al., 2012).

3.3. Abscission and the Final Separation

At the last stage of cytokinesis, abscission, completed primary septum digestion splits the two daughter cells apart, this thought to be driven by turgor pressure (Atilgan *et al*., 2015). The primary septum is hydrolyzed by the digestive endo-(1,3)-beta-glucanase, Eng1p (Martin-Cuadrado *et al*., 2003), and the endo-(1,3)-alpha-glucanase, Agn1p (Dekker *et al*., 2004). A recent study found that Cdc42p is required to recruit Eng1p and Agn1p to the septum, thus directing their proper localization at the division site (Onwubiko *et al*., 2020). The membrane trafficking events during abscission are tightly regulated to ensure complete abscission. Septins complexes are involved in the delivery of the digestive glucanases (Zheng *et al*., 2018). Interestingly, a recently characterized different Anillin homologue, Mid2p is required for this process (Tasto *et al*., 2003). It will be interesting to discover how Mid2p regulates the later stages of cytokinesis. The abscission mechanism is described in Figure 4.

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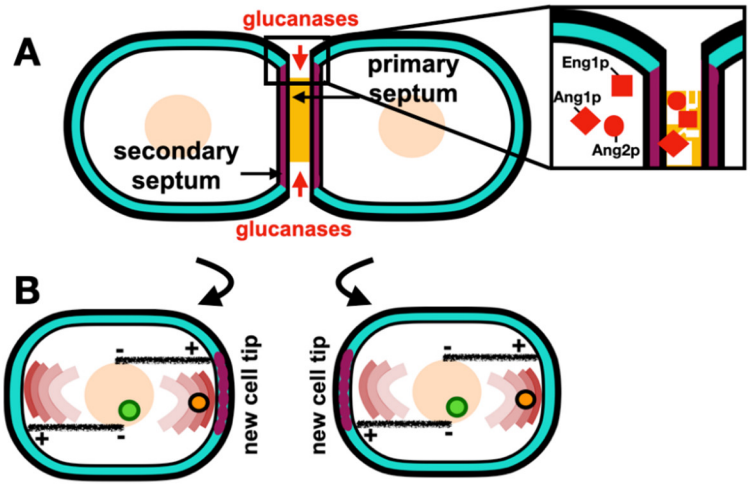

Figure 4. Abscission Leads to the Final Cut during Cytokinesis in Fission Yeast. (**A**) After establishment of the ACR degradation via the ESCRT machinery, glucanases digest the primary septum. (**B**) The secondary septa represent the cell wall material at the new cell tips, next the newly divided cells enter interphase and divide as described in Figure 1 and Figure 2. References within the text.

4. Concluding Remarks

Studying the function of gene products and their localization through the cell cycle in fission yeast has been a powerful way to understand eukaryotic cell division, especially with advanced live cell imaging, fluorescent macromolecules, and the integration of different research fields including cell molecular biology, cell mechanics and mathematical modelling. This enabled the generation of a model of *S. pombe* cytokinesis as described in this review. In summary, a ring first forms at the equator of the cell, and while the new cell-wall material is deposited through membrane trafficking, the ring constricts through a complex network of forces balanced by coordination between ring constriction and septum synthesis. Digestive enzymes then cut the remaining septum material connecting the two daughter cells leading to their final separation. This review addresses the dynamic nature of these processes and presents a visualized picture of current understanding of the proteins and processes that control this important event. It is anticipated that these mechanisms will be conserved across all eukaryotes and so will be informative about human cells and disease conditions, where they are defective.

Conflicts of interest: No potential conflict of interest was reported by the author(s).

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